

# THE BOTANICAL GAZETTE

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EDITORS  
HENRY CHANDLER COWLES AND JOHN MERLE COULTER

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WITH SIXTEEN PLATES AND ONE HUNDRED NINETY-SIX FIGURES



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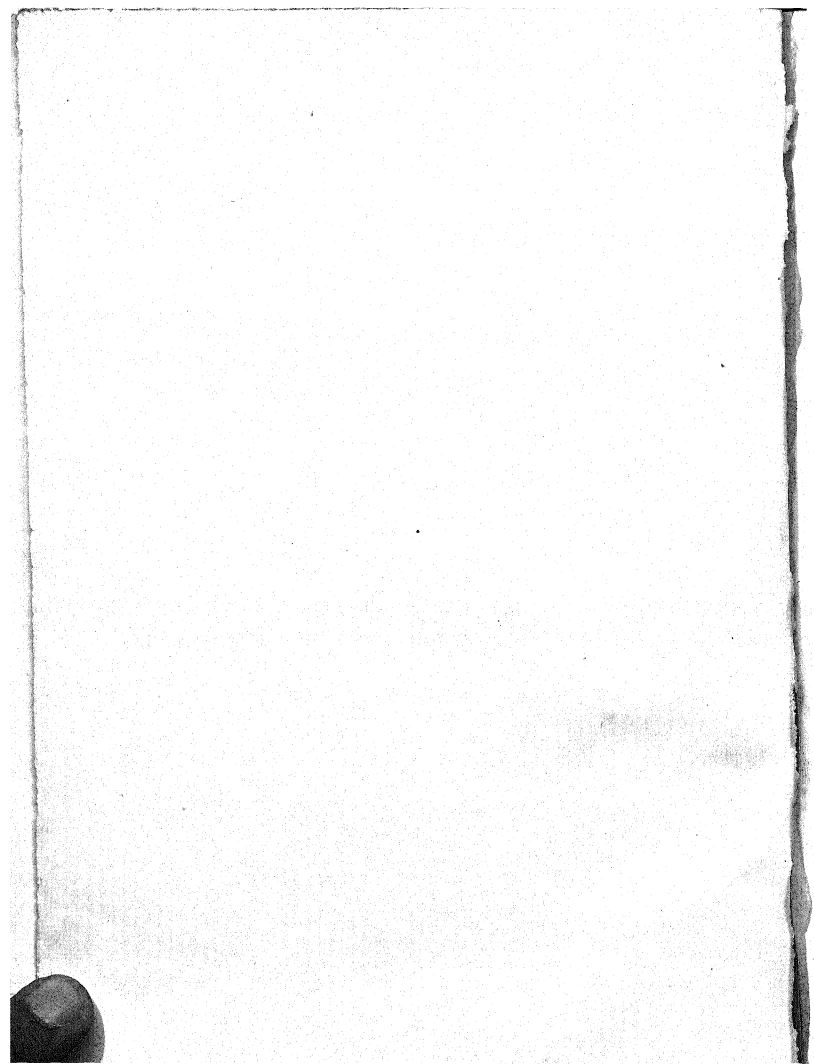
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## ERRATA

## VOL. LXXXV

P. 66, line 2, insert "Funchal" after "Quinta do Deão"

P. 68, line 11 from bottom, for "*Ilex*-covered" read "*Ulex*-covered"



THE  
BOTANICAL GAZETTE

September 1928

ASEXUAL REPRODUCTION IN COLEOCHAETE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 382

OPHELIA C. WESLEY

(WITH PLATES I, II AND FORTY-ONE FIGURES)

Introduction

*Coleochaete* occupies a position of peculiar interest and importance, because of the various stages in the transition from the branched filamentous form to that of the many-celled disk, because of the covering developed from the surrounding cells around the fertilized egg, and because of the cellular body produced by divisions of the fertilized egg. This many-celled body, which is the nearest approach in the algae to the simplest sporophyte found in the liverworts, has attracted much attention in the past. Since no extended and critical work has been done following the classic work of PRINGSHEIM (13) in 1854, JOST (5) in 1895, and OLTMANN (11) in 1898, it seemed worthwhile to cover the genus, as much as possible, both morphologically and cytologically, applying more modern methods of technique and using the latest microscopical equipment.

While it is planned to cover the entire life history of every species of the genus in this investigation, the present paper takes up only that part of the life history from the formation of the zoospore produced by the large thallus to the formation of another mature thallus. Only four species will be considered at this time, *Coleochaete irregularis*, *C. soluta*, *C. orbicularis*, and *C. scutata*. In a paper to follow soon the sexual stages of these four species will be discussed. The material on hand is not sufficient for the different stages of the

entire life histories of *C. pulvinata* and *C. nitellarum*, but it is hoped that such material may soon be available, in order that the study of these two may be completed. So far it has been impossible to obtain material for the study of *C. divergens* and the two Australian species *C. baileyii* and *C. conchata*, so that these too must await further collection and work.

Much of the ground covered by this investigation has been covered before, but in order that the life history might be as complete as possible, it was deemed advisable to reinvestigate the entire life history, hoping to detect any errors that might have crept into the accounts, and to discover such additional facts as had escaped observation. In order to bring all the information into as full and connected a form as possible, it was thought best to give an account of the work done at this time without reference to any previous work. All such work will be given credit in the discussion, and all new work will be listed in the summary, thus making it possible to obtain a full understanding of the genus, even by one entirely unfamiliar with the subject, while one who is familiar with it may obtain the new points without reading the entire paper.

### Materials and methods

Material for this work was collected in a lagoon in Washington Park in Chicago, and in a river at Miller, Indiana. The collections in Washington Park were made weekly from July 6, 1926, to November 30, 1927, after which they were made monthly until April, 1927. The collections at Miller were made at irregular intervals from June 18 until October 30, 1927. The material collected in Washington Park was found growing on *Sagittaria*, while that at Miller grew on *Typha*.

SIZE, CONDITION, AND DISTRIBUTION.—The thalli range in size from one cell to 5 mm. in diameter in *C. scutata*, one cell to 0.065 mm. in *C. soluta*, one cell to 0.06 mm. in *C. orbicularis*, while in *C. irregularis* it spreads in an irregular mass hard to estimate, for often quite long filaments run out from it. The cells vary in size in *C. scutata* from 13.2–25  $\mu$  in width, 15.4–30  $\mu$  in length; *C. soluta* from 7.3–11.7  $\mu$  in width, 8–26.4  $\mu$  in length; *C. orbicularis* from 4.4–10  $\mu$  in width, 8–13.2  $\mu$  in length; *C. irregularis* from 6.6–15.4  $\mu$  in width,

8.8–16.9  $\mu$  in length. *C. orbicularis* can always be distinguished from *C. soluta* by its more regular outline, the dorsal placing of the hairs, and its regular method of cell division, as contrasted with *C. soluta*, whose outline is very irregular, whose hairs arise from the side of the cell, and whose cell wall pushes in from the outside, following the line of division of the chloroplast. *C. scutata* and *C. irregularis* could not be confused with any of the other species.

From the time of the beginning of the collections until November 30, 1926, the thalli increased in size and number, while from that time until the return of warm weather no new thalli were formed, nor was there any considerable growth. From November 30, 1926, until March, 1927, thalli were taken from under and from the ice, and these proved to be in perfect vegetative condition, for the second morning after being brought in they were producing zoospores in abundance; in fact, thalli that had been frozen in the ice for months, when brought into the warm room in February began the production of great numbers of zoospores.

The material was very abundant from just below the water's edge to about 6 inches below the surface. All four species were often found in the low power field. Sometimes the thalli extended farther down, but nearly always in much less abundance. Light seemed to be the determining factor, because a petiole or leaf, as the case might be, had few if any thalli on the shady side, whereas when a plant to which *Coleochaete* was attached stood in the open its petioles would often be covered on both sides.

PREPARATION OF MATERIAL.—*Sagittaria* petioles were split open and placed with the *Coleochaete* side next to a piece of glass, and were scraped with a scalpel, removing nearly all of the cells of the petiole except the epidermal ones. By this means the substratum was made thin enough to stain and still not materially obstruct the view. This method of treatment proved impossible for *Typha*, and all habit material was removed from that with a safety razor blade. Since the thalli of *C. scutata* on *Typha* were especially large this was possible. The thalli became very brittle in the higher grades of alcohol and xylol, hence they were carried through these in glass tubes, one of whose ends was covered with a piece of fine bolting silk. In this way they could be handled with little or no injury.

Material separated from the substratum in this way, as well as pieces of the epidermis of *Sagittaria*, were placed in tap water in large shallow glass dishes whose flat bottoms had been covered with microscope slides. These were then placed in the sun, and as the zoospores were formed many of them settled on the slides and attached themselves so firmly that they were killed, stained, washed, run up, and mounted just like a slide of sections. As the hot summer sun killed not only the zoospores but the mature plants as well, the early fall proved best for preparation of such mounts.

**KILLING AND STAINING.**—Stock chromo-acetic solution, weak chromo-acetic, formalin-acetic-alcohol, formalin-acetic-water, and distilled formalin-acetic-water were used to kill in. Osmic was added to the stock chromo-acetic solution when figures were desired. That killed in stock chromo-acetic proved entirely satisfactory, hence was most used. Fresh material when compared with that killed in it showed the same structure and arrangement of parts of the protoplasm. At all times when possible fresh and fixed material were compared, in order to avoid, as nearly as possible, mistakes due to reactions to the killing agent. Although other stains were used, Haidenhain's iron-alum haematoxylin gave the best details of structure. Power's carmine stain for sporelings gave remarkably clear transparent and well differentiated mounts. Orange G was frequently used as a counter stain.

**MOUNTING AND IMBEDDING.**—The material to be mounted whole was run through the different grades of alcohol and xylol, just as paraffin sections are, and mounted in balsam. Since the pieces of *Sagittaria* and the separated thalli became very brittle and warped, an extra supply of balsam was necessary; therefore some was placed on both sides and a clip attached to the mount to flatten out the piece and get rid of the surplus balsam.

The usual routine method was followed in running up the material for imbedding. Narrow strips of cheese cloth were used to suspend the paraffin in the shell. By this means a piece of paraffin large enough for complete infiltration could be suspended at any depth, thus avoiding crushing the material. After from 4 days to 4 weeks on top of the oven, the material was poured into copper trays  $0.75 \times 1 \times 6$  inches and covered with parawax. This was



changed four times in the 45-60 minutes they were kept on a specially constructed transite box whose temperature ranged from 52° at one end to 70° C. at the other. Thus the temperature was kept at from 52-53° C. during the entire time. The final imbedding was done in paraffin that had been heated to the smoking point and kept there for 6 or 7 hours. This gave a very homogeneous paraffin that could be cut at from 3-10  $\mu$ .

### Zoospore production

FORMATION OF OPENING.—The normal position of the platelike chloroplast is flat against the dorsal wall, covering nearly all of the exposed surface (fig. 2 *X*). Wherever an opening is being formed, the chloroplast is pressed against the wall in the spot just below the place where the opening will appear; the remainder of the plastid stands away from the wall. The plastid at the point of contact shows a much denser, more darkly staining region (fig. 3). The first indication of any opening in the wall is a lighter staining area just above the darker region on the chloroplast. All around this region the wall gives the normal reaction to the stain (fig. 3 *b*). A later stage shows an opening where the light staining area has been. This opening is closed only by a new very thin wall which the cytoplasm has laid down on the outside (fig. 5 *w*). This wall is attached to the lateral walls of the cell. The opening when complete has sometimes a smooth outline and sometimes a ragged one (figs. 4, 5). The opening seems to be due to some chemical reaction, probably enzymatic. Although this was not definitely proved, all indications point to a chemical reaction.

ESCAPE OF ZOOSPORE.—Soon after the opening is formed, the chloroplast moves over to the side wall, the protoplasm contracts, and globules of various sizes begin to appear (figs. 2-5). These globules did not turn black when tested with osmic acid, nor purple when tested with iodine. The chemical nature of these globules was not determined, but there is little doubt that they are stored food that may be used by the zoospore and young sporeling. The mass of the zoospore approaches the opening, forming a peak just opposite it (fig. 5). This peak passes into the opening and becomes more and more attenuated, until it extends completely through the opening.

The part on the outside begins to enlarge as currents of cytoplasm begin to pass through; and the part on the outside increases as that on the inside decreases. The cytoplasm showed currents passing through all the time. The chloroplast passed through toward the last, and soon after that all the cytoplasm slipped through the opening (figs. 6-8). Sometimes the opening is ruptured when the zoospore escapes, but more often it remains intact.

One zoospore whose escape was observed came through in the normal manner, until only a small part of the cytoplasm remained in the cell. The part outside seemed to exert considerable force, while that on the inside failed to slip through easily. The connecting cytoplasm was stretched out into a fine strand until finally, as the small bit slipped through the opening, the strand snapped and the small part was lost to the zoospore (figs. 14, 15). The spore seemed to be uninjured, for after about 20 minutes the cilia appeared and it swam away.

When the thallus is two cells thick the zoospore from the outer cell may escape, and the one from the lower may follow soon after. The contents of the lower cell round up and form the globules just as in the upper (fig. 9). Some kind of pressure seems to be exerted upon the cross wall, which gradually stretches. The spore thus pushes farther and farther into the upper cell, until finally the wall snaps and the zoospore is freed in the upper cell, from which it escapes as did the one originally formed there (figs. 10-12).

Whether an inner thin wall is always formed around the cell contents, closing the opening, or whether it is formed only at times was not determined. All sections through zoospores just before escaping show the opening to be so closed. However, these are either never formed in some cells, are torn off in the escape of the spore, or are torn away in sectioning. Probably the last is the explanation of the fact that often one finds two adjacent cells, one with and one without the remnants of the thin wall and the old cross wall (fig. 12). At times an empty cell showed this thin wall pushed through the pore and open at the end (fig. 13). The lower cell did not always form a zoospore at once, and when this was the case the cross wall became nearly as thick as the outside one.

**TIME OF FORMATION AND ESCAPE.**—There seemed to be no regularity in zoospore formation. In some cases patches of cells were found empty, while in others only a cell here and there had formed a zoospore. As a usual thing only an occasional cell forms a zoospore in the younger thalli, while those thalli that have passed through the winter, in or under the ice, are almost completely deprived of cell contents within a very short time after being brought into the laboratory. Great numbers of adjacent cells form zoospores in a single day, while within a week the thallus would hardly have a cell with its contents remaining. Strange to say, however, not all thalli do this, and weeks after being brought in there were thalli with only a few empty cells. After several days of rapid zoospore formation there gradually comes a lull, and later this is followed by another period of rapid formation. No doubt the change of conditions causes the period of rapid formation, immediately following the change from outdoors to the laboratory.

The time taken for the escape of the zoospore, from the time it starts through until it becomes free, varies from a few minutes to an hour. After the spore becomes free it remains quiescent for 5-20 minutes. At times it rolls over and over in place, and there appears to be some struggle going on; soon the cilia can be seen beginning to wave. First one and then the other begins to move.

The zoospores begin to appear about 7:00 A.M. and continue to escape until about noon. The greater number usually escape between 9:00 and 10:00 A.M. Material brought into the laboratory one morning seldom fails to give abundant zoospores by the next morning, fewer the following day, and so on until zoospore formation almost ceases.

**SPORE.**—The form of the zoospore varies from spherical to egg-shaped (figs. 16, 17); and is the same for all the species studied, save that those formed from the larger cells of *C. scutata* are inclined to be longer and more slender than the others. The size of the spore varies with the size of the cell and with the species to which it belongs. The platelike chloroplast seems to be able to assume any form, for when it occupies the large end of the egglike spore it is cup-shaped; when on the side it is only slightly cupped; while in its normal position in

the cell it is more or less flat. From this one would infer that the plastid lacked rigidity and very definite form, although in general the platelike form is maintained. The chloroplast shows dense and less dense areas, which give it the appearance of being vacuolate (fig. 16). The pyrenoid is not visible in any of the spores studied. Other evidences of the lack of prominence in pyrenoids when the plastid is not functioning rapidly were obtained. It seems quite probable that the pyrenoid loses some of its density when not functioning, and also that the absence of starch grains around it may fail to throw it into such bold relief as to make it noticeable. The vacuolate structure and lack of prominence of the pyrenoid were observed in living material, and also in that killed with osmic acid fumes but not stained. The globules observed in the developing zoospore are likewise to be found here.

The cilia are two in number and of approximately equal length. They seem to arise from the same spot, or at least to leave the membrane at the same place. How they originated was not determined, because of the inability to catch the developing zoospore as it lay outside the pore of the cell from which it came. There is no doubt that the cilia arise during the quiescent period just outside the pore. In all probability they arise from a granule or blepharoplast, although this could not be proved definitely. In order to prove this, some method must be devised by which the spore during the quiescent period can be killed, stained, and sectioned. Granules of various kinds, possibly chondriosomes, were observed at all the stages of spore formation. Many of these were large and dense, as shown by the deep stain. If a blepharoplast is present it could not be distinguished from these granules (figs. 5-11). The cilia are quite heavy for cilia, and are from two to three times the length or diameter of the spore.

**ZOOSPORE MOVEMENT.**—The zoospore moves slowly at first, gradually increasing in speed, although at no time does it move as rapidly as the spores of some other algae. The zoospores move forward almost in a straight line, rotating on the long axis all the time. After rotating in one direction for a time they reverse the direction, and this change invariably comes when the chloroplast side is down. Sometimes the zoospore spins round for a brief time, the cilia seeming

to be entangled; and, after a few seconds, it rights itself and swims away.

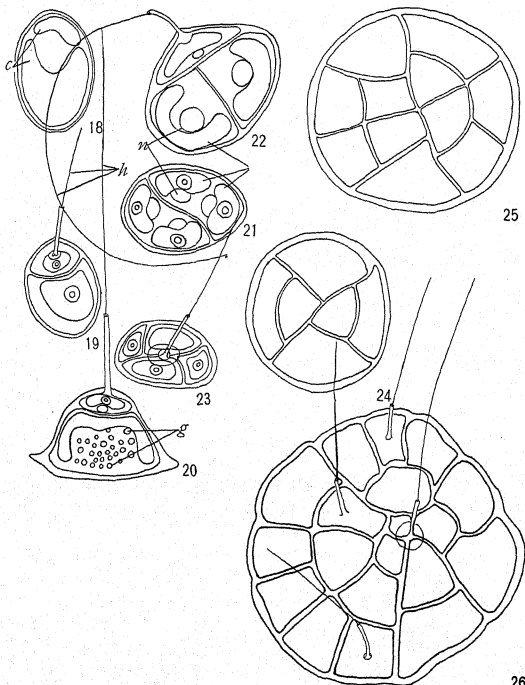
After swimming from an hour to an hour and fifteen minutes, the movement becomes more and more sluggish, and the spore seems unable to manage its cilia very well. They often become entangled, and sometimes one of them could be seen trailing behind the spore. Finally the spore comes to rest with the somewhat larger end down. The cilia are lost, and the wall thickens.

### Development of plant body

EARLY STAGES IN *C. SCUTATA*.—After the thickening of the wall of the zoospore, the chloroplast divides in the usual manner into unequal parts; and likewise the cell divides into an upper smaller and a lower larger cell. The wall is laid down parallel to the substratum. The outer and smaller cell soon forms a hair, and after that never divides or develops any more (figs. 15-20). These changes usually although not always take place in the first twenty-four hours. Next the basal cell becomes very much enlarged and the second division occurs. This wall usually consists of two curves turned in opposite directions, and extends through the long axis of the cell (fig. 21). The depth of the curve varies, while the line of division also varies through an angle of  $45^\circ$ , but nearly always the line passes through the long axis of the cell. At this stage part of the globules are still visible, although before the next wall is completed they have disappeared.

The two cells thus formed may divide simultaneously, or they may divide one at a time. The walls may come in opposite each other, or they may divide unequally, with the small cell of one opposite the large cell of the other (figs. 23, 24).

LATER STAGES IN *C. SCUTATA*.—After the thallus has attained four cells in one plane and has enlarged considerably, cell division continues. The next divisions may be tangential or radial (figs. 24, 25); probably they are more often radial than tangential. If the first are radial and the second also radial, nearly always the next will be tangential. After these first divisions tangential and radial divisions continue, but by no means do they alternate as is shown in fig. 26. Although the divisions are not simultaneous, cell lineage can be traced accurately. As soon as a cell becomes internal, in the sense



FIGS. 18-26.—Fig. 18, zoospore showing division of chloroplast into small upper and large lower ones; fig. 19, two-celled sporeling before growth of lower cell; fig. 20, same after growth: *gl*, globules; *h*, hair; fig. 21, three-celled stage from above, showing lower cells with chloroplasts and nuclei divided ready for formation of new walls; fig. 22, three-celled sporeling from the side, or possibly a five-celled one: *c*, chloroplasts; *n*, nucleus; fig. 23, five-celled sporeling showing large and small cell formed from each of basal cells, dorsal view; fig. 24, sporeling showing that fourth division sometimes forms tangential walls; fig. 25, young thallus showing fourth divisions tangential on one side and radial on other; fig. 26, later stage in body formation, showing relative number and distribution of hairs, also irregular cell division.

that it forms no part of the periphery, cell division ceases; and these cells never divide again unless under the stimulus of reproduction or as a result of injury. Enlargement of the thallus is due entirely to the division of peripheral cells.

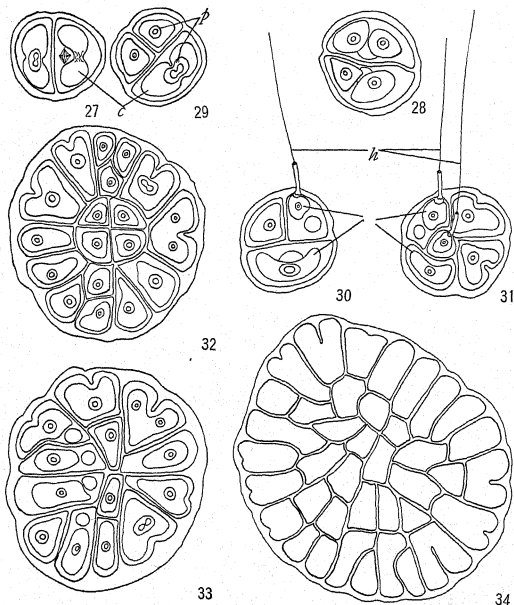
STAGES IN *C. ORBICULARIS* AND *C. SOLUTA*.—The behavior of the zoospores is as described for the entire genus. The first wall laid down in the development of the sporeling is perpendicular to the substratum, and divides the spore into two equal parts (figs. 27, 28). The next two divisions are usually at the same time and at right angles to the first; or sometimes they occur at different times and cut off cells unequal in size (figs. 28-31). So far the sporelings coming from the two species cannot be distinguished.

Even at the three-celled stage hairs may begin to appear, and in *C. soluta* they arise from the lateral wall rather than the dorsal, as is the case in *C. orbicularis* (figs. 30, 31). The peculiar method of cell division, which so clearly distinguishes *C. soluta* from the other species, sometimes begins to show as soon as the sporeling has attained the four-celled stage (fig. 30). In this species the wall, instead of being formed at the time of nuclear division and along the line of the cell plate, as is the usual method, arises as an outgrowth from the peripheral wall, and follows the line of the dividing chloroplast. This chloroplast at such a time often assumes the form, more or less, of a heavy Y-shaped body. The first division in a cell with this kind of a chloroplast usually cuts off one of the upper prongs of the Y, while the following one cuts off the other. In this way not only is the number of rows increased but also the number of cells in the row (figs. 32-34).

In all the thalli of *C. soluta* studied the first and inner cells are closely pressed against each other, and so far as appearance of the relation of cell to cell goes could not be distinguished from those of *C. scutata*. Some of the older thalli showed a very slight space between the filaments making up the thallus, and from the periphery there were short irregular extensions of the thallus, but nothing giving the filamentous character.

In *C. orbicularis* the arrangement of the cells and the method of division are about the same as in *C. scutata*, but the size of the cell is much reduced. The cells divide much more irregularly than in

*C. scutata*, hence the thallus resulting therefrom has the cells more irregular in arrangement and also in form. The mature thallus has a rather regular outline as compared with *C. soluta*.

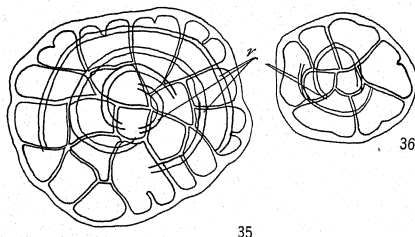


FIGS. 27-34.—*C. soluta* and *C. orbicularis*: figs. 27, 28, two-celled sporelings preparing for second division, and showing nucleus, chloroplasts, and pyrenoids in different stages of division; fig. 29, three-celled stage but showing the other one of first two cells of sporeling in preparation for division. *C. soluta*: fig. 30, three-celled sporeling showing lateral placing of hair; fig. 31, five-celled sporelings showing method of wall formation characteristic of this species, also placing of hairs; fig. 32, young thallus which has arisen from four equal cells formed from zoospore; fig. 33, young thallus formed from four unequal cells developed by division of sporeling; fig. 34, larger thallus showing every stage in cell division, from merest indentation to completed wall cutting off one prong of Y-shaped chloroplast.



As growth proceeds the younger thalli leave broken, more or less regular rings on the surface. These seem to mark the outer limit of successive periods of growth (figs. 35, 36). Whether these lines are purely mucilaginous, or partly or entirely cellulose was not determined. They have the same appearance and react to the stain in the same way as do the cell walls.

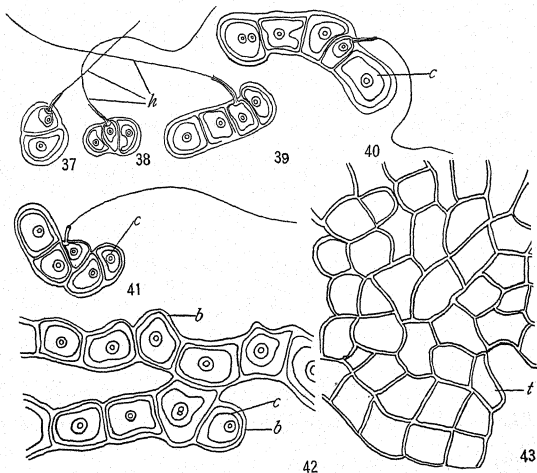
STAGES OF *C. IRREGULARIS*.—The zoospores of *C. irregularis* divide in the same manner as those of *C. soluta* and *C. orbicularis*. Each of the two cells enlarges and divides simultaneously, or at



FIGS. 35, 36.—Young thalli showing rings marking limits of different stages of growth.

different times, or one of them may form a hair before division takes place (fig. 37). More often one cell divides and cuts a small cell which forms a hair and never divides after that, reminding one of the upper cell in *C. scutata* (fig. 38). Fig. 41 shows one with an upper cell bearing a hair, but this was not formed as it is in *C. scutata*; however, the end result was just the same. After the sporeling becomes three-celled, the end cells elongate and divide and the new end cells do likewise, continuing the growth of the filament (figs. 39–41). Fig. 42 shows different stages in the formation of the branches. Any free wall may bend outward and continue to bulge, until it has formed an extension almost as large as a cell. At the same time the chloroplast grows out into this and finally divides along the line of the old plastid, after which the nucleus divides and a new wall cuts off the branch cell, which acts just as any end cell would. Branching is so profuse that the entire surface of the substratum often becomes cov-

ered with cells (fig. 43); in fact, every free surface seems to tend to branch, and the end result in an old plant is a platelike mass showing no tendency to a morphological center. From the outer edge



FIGS. 37-43.—*C. irregularis*: fig. 37, two-celled sporeling showing one with hair (*h*); fig. 38, three-celled sporeling showing center one with hair (*h*); *c*, chloroplast; fig. 39, four-celled sporeling showing one enlarged end cell and one cell with hair; fig. 40, five-celled sporeling showing end cells enlarged and getting ready for division; fig. 41, five-celled sporeling showing production of one hair bearing cell which never developed any more, and the other four cells developing from one of the two first cells of sporeling; fig. 42, young plant showing different stages in branching; *b*, branches; fig. 43; platelike plant body, developed by profuse branching; *t*, thallus.

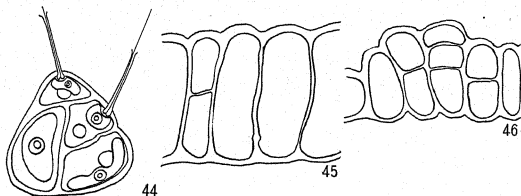
much branched filaments extend in every direction, giving the whole a different appearance from the disk type.

All the species studied save *C. irregularis* may become two or three cells thick, and especially is this true where the thallus grows over an irregular surface. Even very early in the development from the spore this increase in thickness may appear (fig. 44). There

seems to be no regularity to the division of cells parallel to the substratum. Only one cell of a large group may divide, or only one may fail to divide (fig. 45); then again an equal number may divide. More than two cells are seldom formed unless the thallus is growing over a very irregular surface. Fig. 46 shows a thallus three cells thick on a more or less regular surface.

#### Evolution and development of plant body

In the case of *Coleochaete pulvinata* and *C. divergens*, the plants are distinctly filamentous with no tendency to form a plate. In *C. irregularis*, while resembling the other two in being filamentous and



FIGS. 44-46.—Fig. 44; young sporeling of *C. scutata*, showing three cells in thickness, one of which is first cell cut off by spore; fig. 45, section through *C. scutata* showing one cell that has divided parallel to substratum, surrounding ones remaining undivided; fig. 46, section of *C. orbicularis*, showing three cells in thickness formed on regular substratum.

branched, the mature plant shows a distinct tendency to be plate-like, due to profuse branching. There is no morphological center, however, and cell sequence cannot be worked out, but when this is separated from the thing on which it is growing, it holds together in one mass. This plate effect is attained by means of branching, a branch being put forth from every free wall. In *C. soluta*, on the other hand, the branching is attained by a division of an end cell. At first the thallus shows no sign of being filamentous, while later spaces are to be found between the lines of cells; and at the periphery the lines may have very unequal growth, and the outline may be quite irregular. In *C. orbicularis* and *C. scutata* the plant body seems to have become definitely platelike. The growth shows little or no tendency toward the filamentous form, save that growth is

terminal, and that new rows of cells are made by division of the end cell, rather than branching from an old cell. Because of these, the disk might be interpreted as a branched filamentous form that appeared platelike, because it was held close against the substratum by the attaching fibers and because of the profuse branching.

### Hair formation

**FORMATION OF OPENING.**—The opening in the wall through which the hair extends seems to be formed in exactly the same way as that of the opening for the escape of the spore. At any rate, when there is definite indication that a hair is to be formed the pore is already there; and it appears to have been formed in just the same way. Of course, if it were formed in just the same way, one could not tell whether the cell is to form a spore or a hair, save that the opening for a hair is slightly smaller than that for the escape of a spore.

**EARLY STAGES.**—Soon after the opening is formed, or at about the time of its completion, a cell wall is formed just inside the old one. The chloroplast rolls into an incomplete cylinder, with one of the open ends just below the opening in the wall. About this time a dark deeply staining egg-shaped granule is seen, either in the lower part of the opening or in the upper open end of the chloroplast (fig. 47). This granule is suspended in the open end of the chloroplast by means of cytoplasmic strands, at least at times, although they were not always in evidence. Quite often a second and larger granule is found lower down in the space inclosed by the chloroplast, and associated usually with the nucleus. A heavy strand of cytoplasm connects these two granules. Sometimes other granules, less egg-shaped and more nearly round, are seen in the cytoplasm, but always the one, and sometimes the two, are so definitely formed and of such a uniform size and density as to make them quite conspicuous. In the unstained material these showed plainly as highly refractive granules.

**HAIR FORMATION PROPER.**—From the granule in the opening a dense, finely granular stream seems to issue. This was caught in different stages, showing the stream just beginning, just entering the opening, and then extending into the opening or nearly through it (figs. 48-51).

**SHEATH.**—At this time the cone of the sheath is clearly visible. The cell wall formed just before the pore was complete seems to be pushed up into the pore. From the side this gives a conelike outline. It is still in a very plastic state. The stream of cytoplasm seemed to force it into the opening, or perhaps pressure of the cell contents did this; at any rate it extended into the pore and the stream of granules extended through the center, leaving considerable space between the sheath and itself (figs. 48–50). The sheath is forced farther and farther, until it extends for some distance beyond the limits of the wall. As the sheath increases in length the base becomes much enlarged, and extends into the cell. This enlarged part forms an extension of the newly formed wall. At this time the entire sheath becomes thicker, and reacts to the stain in the same way that the normal wall does. When completed the enlarged knoblike base shows an inverted funnel-like opening through which the granular stream passes (fig. 54). The upper end of the chloroplast wraps round this enlarged base.

**HAIR.**—Finally the developing sheath is ruptured at the end, and the finely granular stream continues out into the water. The finely granular cytoplasm may become homogeneous before or after emerging from the sheath (fig. 55). Just what brings about this change was not determined. It may be due to contact with the water, or it may be due to some change within itself. At any rate the hair continues to grow until it has become 3–8 times the length of the sheath (figs. 20, 22).

Usually the hairs project at right angles to the surface. Sometimes, however, immediately after leaving the pore they curve in such a way as to lie flat against the surface of the thallus. Again they may curve so much and so tightly as to form a knot (fig. 57). In a thallus in which the zoospores had escaped from the outer cell, while the one from the lower had not followed, the cross wall became thickened, and the lower cell formed a hair in the usual way (fig. 58). The sheath of this extended through the pore in the wall of the outer cell. A case similar to this was one in which the adjacent cell to one from which a zoospore had escaped formed a hair with the sheath extending into the empty cells. Only two cases of this kind have been observed, and both were killed before the sheath was complete.

What the course of the hair would have been can only be conjectured, but in all probability it would have grown through the pore in the outer wall (fig. 59). Another very peculiar formation was the sheath showing four hairs coming from it (fig. 61). One was found with two and another with three hairs. An abnormal case was one with two granules side by side (fig. 60). The cytoplasmic streams united, and this may be the explanation of the two to four hairs coming from one sheath.

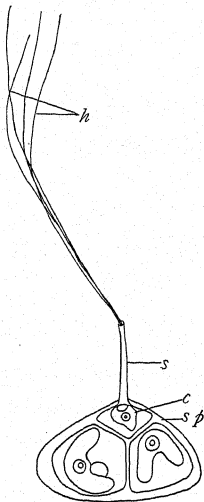


FIG. 61.—Four hairs coming from one sheath; *c*, chloroplast; *h*, hairs; *s*, sheath; *sp*, sporeling.

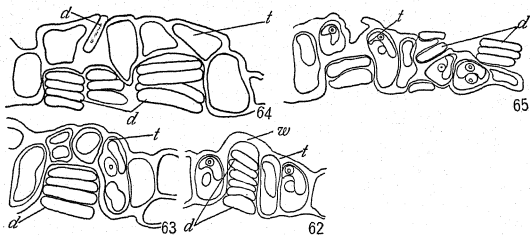
### Relation to diatoms and substratum

RELATION TO DIATOMS.—No instance of the germination of a zoospore on top of a diatom was found, but often the zoospore germinated near them and grew over them. Sometimes there is only one, and at other times there are 4-6 piled one above the other underneath the thallus (figs. 62-65). Usually smaller cells are formed above these, but one was found in which no sign of a cell above the diatoms could be seen. There appeared to be only an extension of the walls over them (fig. 62 *w*). The thallus shows a remarkable ability to adapt itself to the irregularities of the surface over which it is growing. Diatoms were not only beneath the thallus, but

in such great numbers on its top that a surface view of the condition of the thallus was impossible at times. Sections through the plant revealed diatoms almost completely inclosed in the tissue (figs. 64, 65).

ATTACHMENT TO SUBSTRATUM.—Soon after the zoospore comes to rest and begins the formation of its cellulose wall, fiber-like projections begin to form, extending from the wall in the direction of

the surface on which it is resting (fig. 66). In the three-celled stage of the sporeling the fibers seem to grow toward the lateral walls of the epidermal cells, while an older, more mature thallus shows these attaching organs growing down between the lateral walls (figs. 67, 68). Whether these fibers always penetrate between the lateral walls of the epidermis was not determined. One that grew between the guard cells of the stomata was much thickened before it finally reached the wall where it was attached (fig. 69 *w*). It seems quite possible that lack of pressure may have stimulated the cells to produce these, for there is a slight depression where each lateral wall is



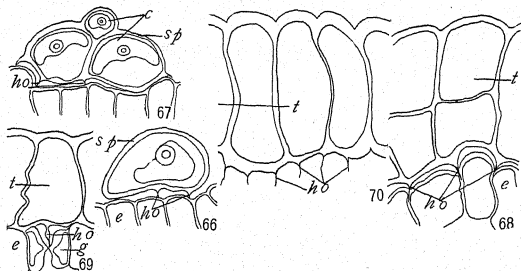
FIGS. 62-65.—Fig. 62, cell wall covering group of diatoms; *d*, diatoms; *t*, thallus; *w*, wall; figs. 63, 64, diatoms underneath thallus; fig. 65, diatoms on thallus and almost inclosed by it.

found. That this method of attachment is quite effective is shown by the force required to separate the thalli from the substratum. One can wipe off the surface with considerable roughness and pressure and make no impression on them. The thalli tear into pieces more easily than separate from the substratum. Fig. 70 shows a portion of a thallus that had grown over diatoms. It had been torn away, leaving the fibers attached to the lower walls. Whether this is the method of attachment to substances like glass was not determined.

#### Aplanospore production

In all the species studied aplanospore-like cells are formed. The contents of a cell seem to have contracted and formed a heavy wall around the entire mass. Inside the wall the cell contents are quite

dense, and filled with small globules much like those in the zoospore but smaller. These are obviously stored food. No chloroplast could be found in any of the sections or whole mounts studied, but thalli from which all the other cell contents have escaped have an occasional aplanospore, which shows bright green, although here again the chloroplast could not be seen (figs. 71, 72). It is possible that these sporelike bodies are other plants that have gained entrance into the cell, but they were found in such abundance in some thalli



FIGS. 66-70.—Figs. 66, 67, one- and three-celled sporelings, showing early development of fiber-like holdfasts; *e*, epidermis of *Sagittaria*; *ho*, holdfast; *sp*, sporeling; fig. 68, portion of thallus showing holdfasts penetrating between lateral walls of epidermis; fig. 69, holdfast developed between guard cells; *g*, guard cells; fig. 70, portion of thallus that developed over diatoms and put out holdfasts between (cutting separated it and revealed the long holdfasts).

that this explanation hardly seems probable. However, these bodies were not germinated, and therefore no definite statement can be made.

### Regeneration

By regeneration is meant here a rejuvenation of the cells around an injured area resulting in the formation of new lobes of the thallus. While *Coleochaete* when injured does not, as do some animals, form new parts to take the place of those removed by injury, it does form new tissue around the injured region. Injury to the thallus of any of the species is quite common, and probably the commonest cause of injury is the snail. These are found in every stage of development,



from the egg to the adult, wherever *Coleochaete* is growing. The contents of any injured cell may either disintegrate within the old cell wall, be diffused into the water, or be consumed as food by the snail. In any case the adjacent cell walls would be exposed to outside influences, and immediately begin to thicken (fig. 73).

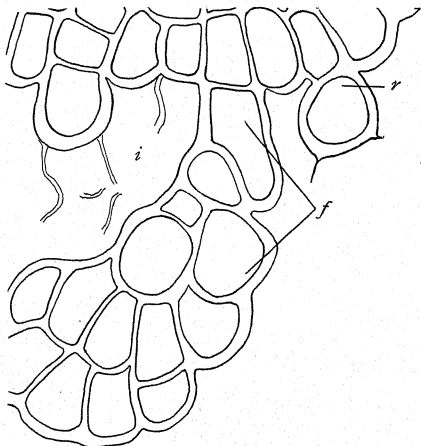


FIG. 74.—Fan-shaped growth developed from rejuvenated cell

Any number of cells around the injured area might show an increasing density and a deeper green color. The exposed walls, as soon as the adjacent cells were removed by injury, bulged out. The walls continue to increase in size, and cell division follows (fig. 71 r). Sometimes only one cell in a place is rejuvenated, while in other places two and even three cells may form the new lobe of the thallus, all three acting in the same way as any three peripheral cells of an uninjured thallus. The method of division and the general appearance of the lobe correspond to that of a portion developed from one to three of the cells of a sporeling. Here, as in the case of normal

growth, new cells are always formed by the division of peripheral cells (fig. 74).

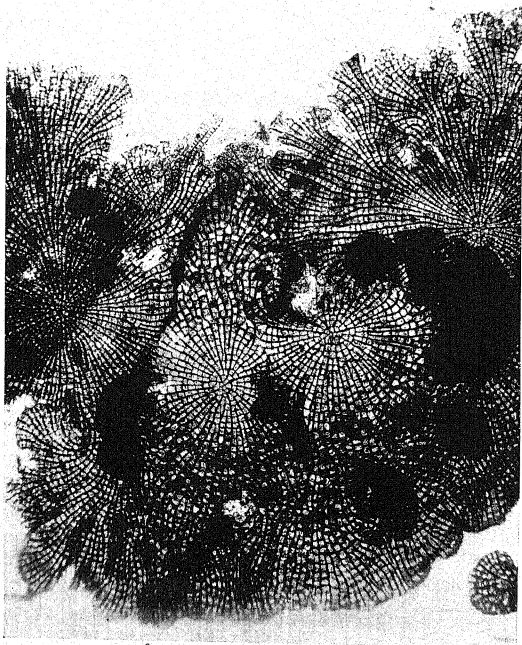


FIG. 75.—Photomicrograph showing ruffled and fluted edge

The species used to illustrate this phase was *C. scutata*, but the general behavior is the same for all the species studied. One thallus observed showed rows of cells 4-6 cells in length, each row being

separated from the other by about the amount of space that some 2-4 cells would occupy. This region was near the center of the thallus and covered about one-half of it. By the bulging thick walls these rows of cells showed that they had been produced by rejuvenation. Some four or five tangential divisions must have preceded the first radial one.

After radial division begins, the new growth follows the normal habit of growth, until soon the cells have grown out and pressed against one another. From this point the thallus has the appearance of any other normal one.

Because of the lack of lateral pressure the new cells spread and continue to spread, until the new part becomes fanlike. In those plants that are injured around the periphery after they have reached maturity, the numerous outgrowths give them the appearance of being ruffled or fluted (fig. 75).

A peculiar thing about this new tissue is its tendency to grow, not only over the old uninjured portion, but also over any other new lobe produced in the same way. In contrast with this, thalli developed from two zoospores that had settled down near each other, form cells that meet in the process of growth and simply flatten their ends against each other; and the next ones do likewise, until the cells are so closely associated that they cannot be distinguished from any two cells of the same thallus. The fact that two zoospores, instead of one, are responsible for the formation of the plate of cells can only be seen because of the peculiar angle at which the cells meet and the two morphological centers. When separated from the substratum the thalli break apart at any other place as easily as along the line of union.

Another interesting thing to be noted in this connection is that when fruiting begins in two thalli that have grown together in this way, the first antheridia are always formed just back of the line of contact of the two.

#### Discussion

The work of PRINGSHEIM on five of the nine species of this genus laid down in a remarkably clear and accurate manner the broad outlines of the genus. In spite of his excellent work, however, some of the finer details were omitted. PRINGSHEIM (13) described accurate-

ly the change in position of the chloroplast in zoospore formation, also the appearance of the globules or droplets. As to the actual manner of escape of the spore, he merely noted that it escaped through either a break in the wall or a pore. The formation of the pore prior to the change in position of the chloroplast was not noted. This pore was evidently the result of some chemical change brought about by the chloroplast. More than likely it was made by an enzyme produced by the chloroplast. The appearance of the change, as well as the outline of the region of change, seemed to point to this.

A very interesting question was why the spore forced itself through the narrow pore. According to JOST (5) the zoospore escapes because of the swelling of the inner membrane. He also claims that they will not escape if kept in the dark, and reports that the first division of the zoospore which had been kept in the dark occurred without shedding. Nothing in any of the spores studied indicated a swelling of a sheath or anything of the kind, while the effect of light was not tried. The shrinkage of the cell contents seems to preclude the idea of pressure from within, while no cause for a lessening of pressure from without could be found. The spore passed through by an amoeboid movement, and probably went through the opening because that was the only place where it was not stopped by the cell wall. It seems quite probable that the movement was entirely without direction, save for the light stimulus that JOST speaks of. The movement of an amoeboid mass across a glass slide when mounted in pure water must be purely aimless. It will crowd between any obstacles in its way in the same manner that the spore goes through the pore. Although the spore does not put out pseudopodia, save the extension that takes it through the opening, it seems probable that its passage is quite similar.

The only reference to the time of production of zoospores is that by JOST, who says that they escape in the early morning hours. No one gave the time, or behavior of the young spore, hence a record of the exact time as well as the early behavior was made.

The form, light refracting globules, and the number and position of the cilia observed by former investigators were confirmed by this work. The only point of departure of the two accounts consists in the description of the chloroplast as given here. The movement of

the spore had been accurately described by PRINGSHEIM and JOST, but some minor details, together with the length of the period of movement, were not given by them, nor did they mention the quiescent period.

Their account of the development of *C. scutata* differs from mine only in one point: the double curved line of the second division by the sporeling, instead of being the exception, or one of the forms of division, as given by JOST, is almost the only kind in the many hundreds observed by the writer. Of course there is quite a wide variation of the angle that this line makes with the long diameter of the cell, but it most often runs through the longest line.

In their accounts of the development of *C. soluta* and *C. orbicularis* there is a wide divergence from this account. They get two cells from the zoospore just as I do, but PRINGSHEIM then gets an outgrowth in the form of a curving branch from each of these two cells, and these branches put out other branches from which the filaments arise, while the material used for this work showed the first two cells enlarging and dividing into two equal (or large and small) cells. Both were found frequently, but not a single instance was found of the kind reported by PRINGSHEIM, in spite of the fact that literally hundreds of germinating zoospores were examined. After the formation of the first four cells, they enlarge and new cells arise by their division. After the second circle of cells is formed, division of the peripheral cells by growth of the wall from the outside in causes the growth of the thallus in the case of *C. soluta*. It is hard to account for this difference, since one could hardly mistake this for any other species. Of course it is possible that this is a variation of the same species, or that the difference in environment causes the variation in development. Such a careful observer as PRINGSHEIM could hardly have been mistaken in what he saw. On the other hand, check and recheck with his results in mind have always yielded the same result.

LAMBERT (6) reports that the plant body is 1-3 cells thick, while PRINGSHEIM gives it as one cell in thickness. My observations confirm LAMBERT's account. Just why the thallus should vary along this line is hard to understand. It cannot be due to growth under pressure, resulting in cells too large to take in food properly, for very

often only one cell will divide parallel to the substratum, while all the surrounding cells will remain undivided, in spite of the fact that they are as long and are often broader than the one dividing. On the other hand, all of a group of cells may divide save one or two. Thalli three cells in thickness are rare; most of those were observed where the plant grew over a very uneven surface.

According to PRINGSHEIM the hair was formed by a pushing out of the wall into a long hollow cylindrical sheath, which finally opened at the end and a bristle protruded therefrom. One end of this bristle remained deeply imbedded in the sheath according to the account. He speaks of separating walls but concludes they are really not cellular hairs. MOEBIUS (9) says that the cell pushes out a thin cone into which the lumen extends. The outer membrane enlarges and detaches itself from the inner. Finally, after a further extension of the sheath, the hair breaks through. This was still surrounded by the inner very tender membrane, and because of its cytoplasmic contents was capable of elongation until it was many times as long as the sheath. According to MOEBIUS the content of the lumen of the hair was homogeneous, and there was no aggregation of cytoplasm at the tip. At intervals along the hair he found small light refracting bodies, but concluded they were not cross walls. He also observed a strong light refracting body at the place of origin of the hair in each of his two Australian species.

As will be seen from this account, there is a wide divergence between their findings and mine. The "strongly light refracting body" of MOEBIUS is, no doubt, the dark staining dense granule from which the hair originates. That, together with the fact that the hair consists of a sheath and hair, are about all there is in common between the two accounts. The facts were given in the body of the paper and need not be repeated here, but a few words of interpretation may be given. That the pore is formed by an enzyme secreted in the same way as in the case of the pore of the zoospore seems entirely plausible, since no sign could be found of the formation of the pore in any other way, and cells were found forming pores as in the case of the zoospores. The pore differs in no way save that it is smaller than the other one.

The dark staining, light refracting bodies are blepharoplasts,

chondriosomes, or granules, as one chooses. These granules, as I shall call them, cause a stream of finely granular cytoplasm to issue therefrom. About this time the increased pressure causes the new very elastic wall to push out into the pore. The stream of cytoplasm goes into the center of this elongating membrane and keeps pace with its development. As this goes into the narrow opening the stream is checked, and there is deposited at the entrance a mass of dense cytoplasm. The cell membrane, because of lack of pressure on the outside and pressure on the inside, continues to extend until it reaches the limit of its elasticity, at which time it bursts, and the stream of cytoplasm which has kept pace with it may pass out into the water, and continue to pass out until it has reached its normal length, which is several times as long as the sheath. Either the water or something else causes the granular cytoplasm to become homogeneous and brittle, and the hair is formed, or the granular cytoplasm merely changes itself. More than likely it is the latter, for often the cytoplasm is seen to take on the hard brittle form before leaving the sheath. At the same time that the hair is forming, the sheath and the wall that formed it are thickening and the base of the sheath is becoming much enlarged and thickened, just as if the cytoplasm had been checked at the entrance to the pore and had deposited cellulose there, and more cellulose, until it extended out into the cell like a delta into a bay. That the cilia are formed by the same body or bodies, and in the same way as the hair itself is formed, seems not entirely improbable, since no sign of cilia could be found in either fresh or stained zoospores until toward the end of the quiescent period after their escape. The spores when observed at this time showed no sign of them, when suddenly they were seen waving in the water. The granules have all the appearances and characteristics of a blepharoplast. In order to prove this, it would be necessary to devise some method to catch these spores at the quiescent stage, and to cut and stain them. So far they have eluded the writer.

Former accounts of the genus have made no attempt to explain how the plant body is attached to the plant or other object upon which it grows; they contented themselves by saying that it is attached.

Under the heading Regeneration, growth after injury was discussed. According to McCALLUM (8), regeneration is not really different from ordinary plant growth. His work indicates that the growth activity was not stimulated by anything sent out due to wounding, for he found that the growth was started when the parts, instead of being removed, were only prevented from functioning. His conclusion was that a functioning organ in some way inhibited other potential growing parts from functioning. This may explain why growth occurs after injury of the peripheral cells, but cannot explain growth due to injury of internal cells, as those shown in fig. 71. In this case cells that had definitely matured, and that under normal conditions would never grow again unless to develop antheridia, now after death of adjacent cells became active. Only outer cells of this plant ordinarily divide, but any cell around an injured place may begin to divide and form a new fan-shaped part, in whose peripheral cells only rests the power of division. McCALLUM's reasons certainly do not apply here, since not only are the peripheral cells intact but they are functioning as well. Why then is growth begun here? It cannot be due to release of pressure, because if that were so all the cells would grow, and that is not the case. Why then do some of these cells grow and others do not? Each one has an equal chance for food, water, light, and is exposed to the same temperature. The difference must be in the cells themselves. It is quite probable that all the cells of a thallus of this kind are not in the same physical condition, any more than all the members of the same family are. Those in the better condition recover from the shock of injury, and the lack of pressure on the outside causes the cross walls to push out, due to pressure within. The cell contents increase to keep pace with the increasing space, and growth begins. Since these cells started growing first, they might inhibit the others or might deprive them of available food. This latter does not seem at all plausible, because each cell has equal access to the supplies necessary for growth. Why then do some cells grow while others do not? All peripheral cells grow, and in a way these have all become peripheral. It seems to make no difference whether the cells that grow extend from the peripheral side in, or from the central or lateral sides. Here, if anywhere, the cause of growth following injury should be determined, because here is found about the simplest expression of it.



### Summary

1. The opening through which the swarm spore escapes is made by some chemical change, probably enzymatic, which is initiated and controlled by the chloroplast.

2. Zoospores escape by an amoeboid movement through a pore.

3. There is a period of quiescence after the escape of the spore, at the end of which the cilia appear.

4. The second wall in the sporeling of *C. scutata* is almost invariably a double curved line whose openings are turned in opposite directions. This wall passes through the long diameter of the cell.

5. The first two cells of the sporeling of *C. orbicularis* and *C. soluta* divide to form four or six cells. By division of these the thallus is formed.

6. *C. soluta* is developed into a thallus, with no indication of the filamentous character except the small space between the outer cells of the mature thallus.

7. Hair formation is initiated by a stream of cytoplasm issuing from one or two granules, found either in the lower part of the pore or in the outer open end of the cylindrical chloroplast.

8. The sheath is formed by an extension of the new inner wall which is forced through the pore. This sheath develops a knoblike base, around which the chloroplast is wrapped.

9. Thalli are attached to the substratum by small outgrowths from the basal walls.

10. Diatoms may be overgrown by the thallus, they may cover it, or they may be almost completely inclosed by it.

11. Injury may result in fan-shaped portions developed anywhere around the injured region.

This investigation has been carried on under the supervision of Professor CHARLES J. CHAMBERLAIN, to whom I am indebted for valuable advice and criticism. I am also indebted to Professor W. J. G. LAND for helpful advice and the photomicrograph.

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## EXPLANATION OF PLATES I, II

All figures were drawn with the aid of camera lucida Spencer apochromatic objective 3 mm. N.A. 1.40, in combination with compensating ocular  $\times 15$ , giving a magnification of  $\times 680$ ; except figs. 14-17, which were drawn with Spencer apochromatic objective 1.5 mm. N.A. 1.30 with compensating ocular  $\times 20$ , giving a magnification of  $\times 1775$ .

## PLATE I

## COLEOCHAETE SCUTATA

FIG. 2.—Surface view of two cells: upper cell showing platelike chloroplast curled up at corners, otherwise normal; lower cell contents rounded up to form zoospore: *c*, chloroplasts; *gl*, globules.

FIG. 3.—Section through portion of thallus, showing beginning of pore formation: *c*, chloroplast; *n*, nucleus; *po*, light area where pore is formed.

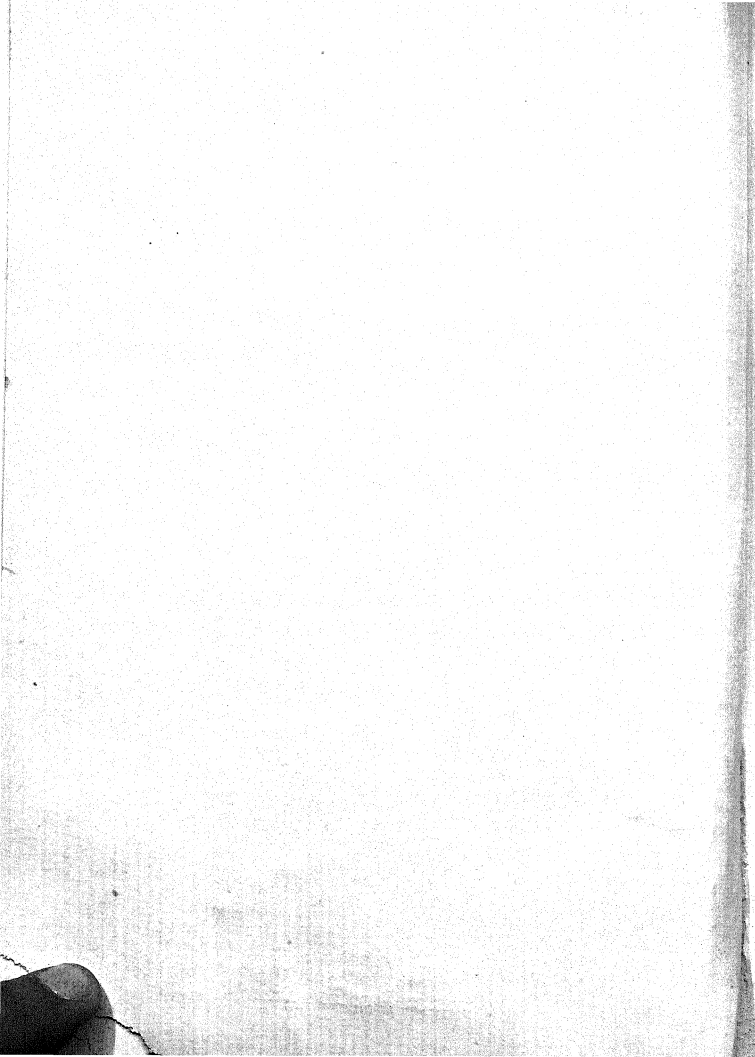
FIG. 4.—Section of thallus, showing completed pore (*po*), cut just to side of pore, showing its regular outline.

FIG. 5.—Section showing zoospore beaked ready to escape and irregular pore closed by new wall.

FIGS. 6-8.—Stages in escape of zoospore.



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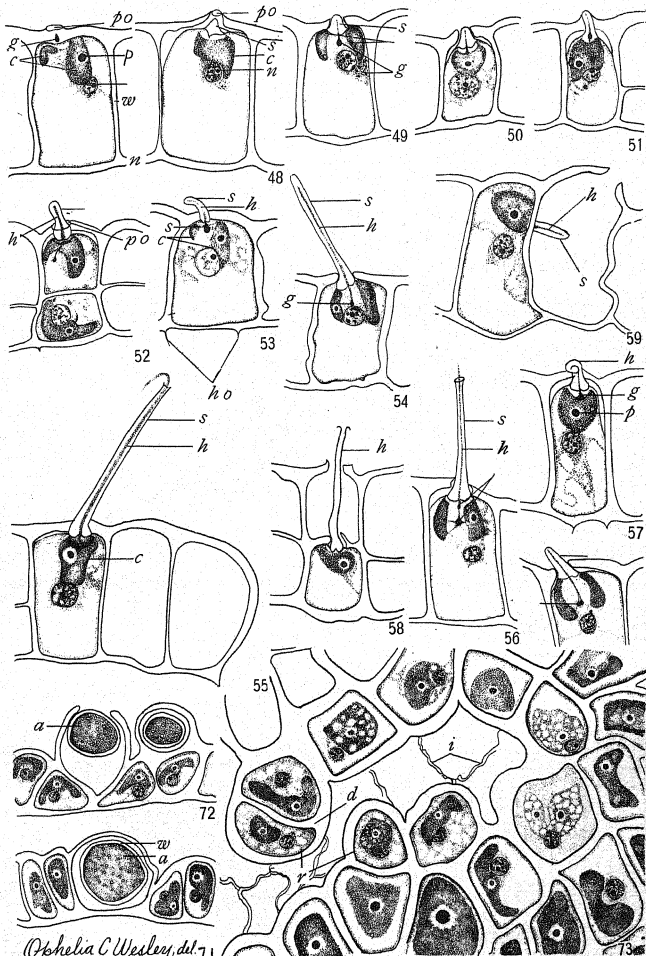




FIG. 9.—Section showing empty cell from which zoospore has escaped and lower cell with contents rounding up to form zoospore.

FIGS. 10, 11.—Stages in escape of spore from lower cell.

FIG. 12.—Cells after escape of spores, showing remnants of new wall, outer one, and inner cross wall which has been much stretched and finally ruptured in escape of the spore from lower cell.

FIG. 13.—Section just to the side of the ruptured new wall, showing it stretched beyond the pore.

FIG. 14.—Escaping zoospore in which the last bit of protoplasm did not slip through pore, and connecting cytoplasm drawn out into long strand.

FIG. 15.—Spore just as the bit of cytoplasm slipped through pore, showing strand snapped in two.

FIG. 16.—Egg-shaped spore: *c*, chloroplast showing dense and less dense areas; *ci*, cilia; *z*, zoospores.

FIG. 17.—Zoospore more nearly round, showing nucleus and not showing chloroplast.

#### PLATE II

##### COLEOCHAETE SCUTATA

FIG. 47.—Section through cell, showing beginning of hair formation: *c*, sections of chloroplast; *g*, granule; *n*, nucleus; *p*, pyrenoid; *po*, pore; *w*, wall.

FIG. 48.—Section through cell, showing beginning of sheath formation: *c*, chloroplast in cylindrical form; *n*, nucleus; *po*, pore; *s*, sheath.

FIG. 49.—Section showing beginning of cytoplasmic stream from granule inclosed by chloroplast: *c*, sections of chloroplast; *g*, granules; *s*, sheath.

FIG. 50.—About the same as fig. 49 except that chloroplast not sectioned but shown entire and hiding granule; depression in sheath being formed.

FIG. 51.—Section of cell showing one granule just below pore, and a second down inside chloroplast which is in usual cylindrical form; sheath formation clearly shown.

FIG. 52.—Section of cell just above pore, showing sheath extending through it; one granule concealed by chloroplast while other revealed because a piece of chloroplast is cut away: *h*, hair, *po*, pore; *s*, sheath.

FIG. 53.—Section showing granule suspended in open end of chloroplast: *c*, parts of chloroplast; *cs*, cytoplasmic strands; *h*, hair, *s*, sheath; *ho*, holdfast.

FIG. 54.—Sheath nearly ready to burst.

FIG. 55.—Completed sheath with stream of cytoplasm extending into water.

FIG. 56.—Section showing upper smaller and lower larger granule.

FIG. 57.—Hair twisted into knot.

FIG. 58.—Hair formed in lower cell and extending through pore in upper.

FIG. 59.—Hair extending from adjacent cell into empty cell.

FIG. 60.—Two granules contributing to hair.

FIGS. 71, 72.—Aplanospores, showing much thickened wall and globules.

FIG. 73.—Injured area inside thallus, showing dead cell walls: *w*, rejuvenated cell and not rejuvenated cell after one division; *r*, thickened walls around injured portion.

## CERTAIN FOSSIL PLANTS ERRONEOUSLY REFERRED TO CYCADEOIDS

G. R. WIELAND

(WITH FOURTEEN FIGURES)

Just as the fine Colorado cycadeoid found by FERDINAND V. HAYDEN and originally called *Zamiostrobus mirabilis* by LESQUEREUX is now recognized as one of the handsomest pygmic trunks in the silicified group,<sup>2</sup> and just as there are occasional fruits or parts of fruits and even foliage which if better known must fall within the Hemicycadales, so there are on the contrary a few fairly conspicuous and long known fossils which require removal from the cycadeoid synonymy. Among these are especially the *Cycadeoidea abiquidensis* Dawson, which is some early araucaroid cone, or else a *Lepidostrobus* or *Lycostrobus*. Also, there is the singular strobilus or head from the Cretaceous formations of Kansas, originally described by LESQUEREUX as *Williamsonia elocata*, which at least merits a revised description, fortunately made practicable by new material in hand.

Moreover, it appears that the specimens from the North Carolina Trias first referred to *Zamiostrobus* by LESQUEREUX, and then transferred to the cycadeoid stems by WARD, must really be of an araucaroid nature. So far no instance is recalled of confusion between araucarian and cycadeoid cones, although it can be seen that such might arise in somewhat imperfectly preserved specimens; for if in araucarian cones the sporophylls became increasingly sterile, or if in cycadeoid cones the organs called interseminal scales (which are but reduced megasporophylls) became increasingly fertile, a certain similarity of outer form would be reached.

As field exploration is extended, and as the study of plant imprints and casts is made more exact, imperfectly known types reach a larger interest. Too often highly significant fossil plants are so meagerly described or illustrated that they remain virtually un-

<sup>2</sup> American fossil cycads. Vol. II. p. 109. pl. I. Publ. 34, Carnegie Inst. Wash. 1906. 1916.



known to botanists and collectors, and so the search for supplementary or better preserved material that should follow may be delayed indefinitely; or new finds may meet the fate of the initial ones. Surely, as the years pass, hypothetic types of fructification on the borderland between cycads and cycadeoids, and particularly between cycadeoids and the early angiosperms, must be found in increasing number.

Accordingly, in here bringing into view a few types now better understood, along with other forms still uncertain, it is not so much mere points in synonymy that are aimed at; the greater object should be to search out the unusual types because of their suggestiveness in evolutionary study, and keep them assembled. By following that method, types of unusual feature long overlooked or sequestered in minor collections are brought to notice, and unexpected results sometimes appear. Thus while attempting to learn at first hand the characters of some of the little known cycadeoids, a request was made to the custodians of Washburn College, Topeka, Kansas, for permission to examine the isolated type of *Cycadeoidea munita*. Long unseen, this type was supposedly in the collections of that college, but search did not at once reveal it. Instead there was first found and forwarded for study the unique fruit mold described later as *Williamsonia* (?) *hespera*. Another of these debatable types is the so-called *Cycadeoidea abiquidensis* Dawson.

ARAUCARITES (*Cycadeoidea*) EMMONSI (emend.). Fig. 1.—EMMONS, American Geology. p. 123. fig. 92a, 1857 ("trunk of a cycad"); FONTAINE, Older Mesozoic Flora. p. 117. pl. LII, fig. 5 (1883) ("might be called *Zamiostrobus emmonsii*"); WARD, Status of the Mesozoic floras. p. 302 and pl. XLIII, fig. 3. 1900 (*Cycadeoidea emmonsii*); WIELAND, American Fossil cycads. p. 6. 1906 ("trunk of a cycad"); KNOWLTON, Catalogue of Mesozoic and Cenozoic plants of North America. p. 214. 1919 (*Cycadeoidea emmonsii*).

The type here renoted was found by the well known geologist EMMONS in the Triassic (Rhaetic) of North Carolina, over 70 years ago, and is now in the Emmons collection of Williams College. It consists of an ornate, more or less carbonized imprint of small spirally set rhombic scars borne on a thin slab of light colored shale, on the obverse of which is seen an impression of a broad cycadean

leaf. EMMONS called the specimen the "trunk of a cycad"; but later FONTAINE considered it to be a cycadeous strobilus, remarking that it could not be a *Lepidodendron*, because it was "a plant which did not exist in the Mesozoic" (!).

In 1894 WARD first referred this fossil to *Cycadeoidea*, refiguring it, and as just noted, giving an extended description six years later.

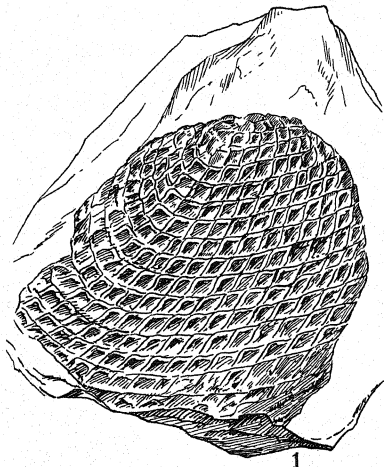


FIG. 1.—*Araucarites* (*Cycadeoidea*, *Zamioctrobus*) *emmonsii* (Emend. Fontaine): cf. with figs. 2, 11, 13; original type from North Carolina Trias (Rhaetic), now in Williams College collection; about natural size.

He is then convinced from an examination of the original specimen that it is the "trunk" of a *Cycadeoidea*, and even suggests some succulent, little woody type, easily crushed. The excellent drawing given is improperly oriented, FONTAINE (1883) having placed as basal what is obviously the apex, although rightly suspecting the fossil to be a cone. It is only 4.5 by 6.5 cm. in size, and might be compared with the English Jurassic cone *Araucarites ooliticus*.

Taking WARD's description and figure, it is certain that this is some kind of araucaroid cone which must be removed from the synonymy of the cycadeoids. He speaks of "ramental walls," but no ramentum was definitely observed, and both FONTAINE's drawing and the better one given herewith show division lines between the scars. Such seldom appear as a persistent limit of the

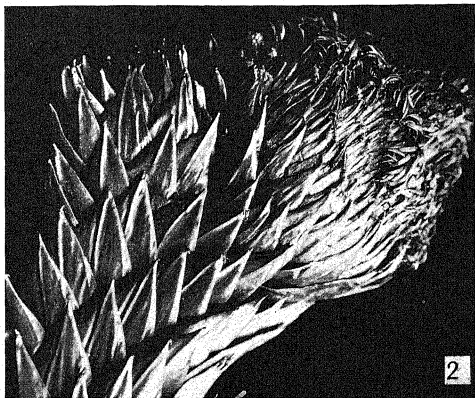


FIG. 2.—*Araucaria imbricata*: one-third to half-grown ovulate cone from Upper Bio Bio Valley, Chile; note on left young vegetative bud, also unwilted straplike ends of megasporophylls, fertile and barren; not oriented; diameter 8 cm.

ramentum borne by adjacent leaf bases. Nor would the ramentum be borne on one side of the bases only. Only in thin sections of petrified material are such lesser features of smaller organs apparent. The lines or "walls" must be merely boundaries between the scales of a cone.

An instance where the scale tips are indicated (compare the present fig. 2) is noted in the clearer araucaroid cone imprint called *Zamioctrobus virginianensis*,<sup>2</sup> which should likewise be transferred to

<sup>2</sup> FONTAINE, pl. XLVII. figs. 4, 5. 1883.

*Araucarites*. Preservation extending farther out, the scalelike character is more obvious. Also such araucarian cones destined to fossilization would be mostly immature, for at maturity the scales split apart, and fall to the ground to become one of the most frequent of fossil seed and scale types.

FONTAINE himself later says of this fossil (WARD's Status, p. 301. 1900): "Examined closely with the help of a lens the leaf scars are seen to be of the same character as those of *Cycadeoidea emmonsii*, but decidedly smaller." Doubtless a younger cone!

The name *Zamiostrobus* is an old one given by ENDLICHER, not as satisfactory for this fossil as *Araucarites* Presl, queried if need be. As an araucarian cone, 5-6 cm. in diameter, with the base incomplete, the features are readily reconciled with a quite cosmopolitan series of Mesozoic fossils of similar aspect. Nor is this the first instance of suggested transfer, *Araucarites* having been used by STERNBERG, PRESL, and later authors, "for cones more like those of *Araucaria* than any other conifers."

Furthermore, the huge and more correctly named cone from the North Carolina Trias given in the "Older Mesozoic Flora" on pl. LII, figs. 4, 4a, as *Araucarites carolinensis*, in connection with the illustration of the *Araucarites* (*Cycadeoidea*, *Zamiostrobus*!) *emmonsii*, suggests some partly lepidodendroid features. Such cones could well pertain to the fine stem fragment of pl. XLVIII, fig. 5, of that work.

In bringing *Araucarites emmonsii* and *A. virginiensis* into araucarian association, there yet remains somewhat the same risk as incurred when *Zamiostrobus* was removed to *Cycadeoidea* without first securing some one critical structural detail. Admittedly the time has come when fossil plants cannot be transferred at will from genus to genus on the basis of outer features alone; or worse, the accumulation of debatable points at great length. It is hoped, therefore, that some one at Williams College may attempt a more careful examination of this fossil plant. But it is not this fossil alone which needs study; the series of Rhaetic plants from Virginia and North Carolina should be restudied in entirety, in both the field and laboratory.

At the time coal mining was carried on at Lorraine, Virginia, and

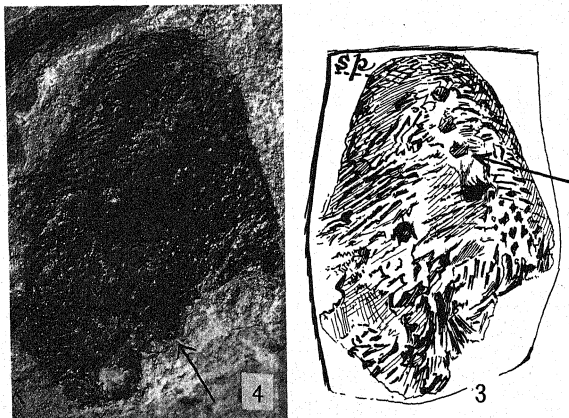
at Egypt, North Carolina, some 40 to 60 years ago, a more remarkable flora was brought to view than could have been obtained by mere exploratory effort. The fossils occur in fine shales, and are nearly always well carbonized, but when they were first studied the only successful closer examination of such plant remains recorded was that of BORNEMANN (1856). Had the chemical methods of imprint study practiced in the past ten years been used on the freshly uncovered Virginia and North Carolina fossils, much more light would have been thrown on this excellent vegetation of the North American Rhaetic, or, as since suggested, Keuper.

ARAUCARITES (?) (*Cycadeoidea*) ABIQUIDENSIS (emend. Dawson). Figs. 3, 4.—DAWSON, J. W., and HARRINGTON, B. A., Geological and mineral resources of Prince Edward Island. p. 45. pl. III, fig. 29. 1871 (*Cycadeoidea abiquidensis*); WARD, L. F., Fossil cycadean trunks of North America. Proc. Biol. Soc., Washington, Vol. IX. p. 87. 1894 (*Cycadeoidea abiquidensis*); WIELAND, G. R., American fossil cycads, Vol. II, p. 109. 1916 (*Cycadeoidea abiquidensis*, of doubtful position).

Professor ADAMS of McGill University some time ago considerably loaned the original Dawson type of "*Cycadeoidea abiquidensis*" for restudy. The Prince Edward Island rocks from which this fossil comes are no longer regarded as Triassic but Permian, from the presence of the Theromorph reptile *Bathygnathus borealis* Leidy (1854), as well as from the geological relations, and in general the plants. While the plant preservation is but fairly good, owing to the prevailing rather granular matrix, considerable carbonization is noted, and further collection afield with study of chemical methods is certainly practical.

The Dawson type comes from the Gallows Point neighborhood of Prince Edward Island, and other examples must doubtless be found. The fossil is certainly not the trunk of any cycad, but a very interesting cone primarily referable to the Araucaroids. As the illustrations show, this cone is preserved in a much flattened carbonized condition. It is about 6.5 cm. long by 4 cm. in diameter, and the photograph (fig. 4) shows the form and features quite as well as they can be shown, unless it should be possible to learn some further facts from successful chemical study. On close scrutiny it

is seen that the organs are set in a pronounced spiral order. Particularly in the area near the summit (fig. 3 *sp*), made from the original Dawson figure, the rhombic pattern of the spirals is un-



FIGS. 3, 4.—*Araucarites* (?) (*Cycadeoidea*) *abiguidensis* (Dawson) from Permian beds of Gallows Point, Prince Edward Island; type in collections of McGill University; slightly enlarged. Fig. 3, drawing made from Dawson's original figure of *Cycadeoidea abiguidensis*, so-called, bringing out certain minor and probably accidental markings mentioned in text; arrow denotes these markings, and at *sp* the sporophyll spirals are distinct, as they are over a much larger area than shown. Fig. 4, photograph of original type; arrow shows position of cone axis. This is a crushed though handsome carbonized cone, with sporophyll spirals mostly crushed down. Toward cone apex the rhombic outlines of sporophyll summits and their spiral succession are distinct (length 6.5 cm.).

mistakable. On the specimen itself the spirals can be traced much farther, although in the flattened position.

The position of the base of the central axis is indicated by the arrow in fig. 4. The rhombic ends of the spirals are small, only several millimeters on the faces, and scarcely as large as in a com-

parable Rhaetic cone called by NATHORST *Lycostrobus scotti*. But as SEWARD says (*Fossil plants*), this cone is little different from *Lepidostrobus*.

Preservation would have to be better to decide whether the present cone is araucaroid instead of lepidodendroid. Certainly if Araucarians were derived from, or in any way related to the *Lepidodendron* complex, somewhere about Permian time the transition forms would have borne cones in all outer features very much like those here seen in outline only.

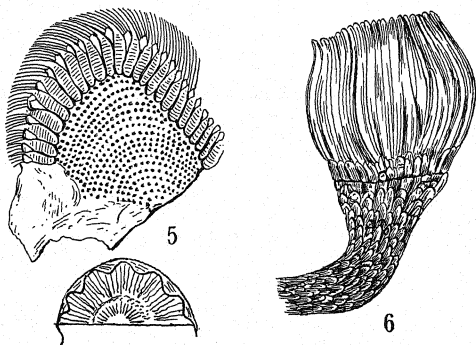
Another feature of the Dawson type is even more uncertain. Scattered about the surface are half a dozen slight bosses, a few millimeters in diameter, indicated in the original drawing and commented on. They scarcely show in the photograph, and are made unduly prominent in the old drawing, as indicated by the arrow. These markings are not branch scars, and certainly are not fruits laterally borne like those of *Cycadeoidea*. They are not in subspiral succession, and cannot be seeds. The best explanation is that they are mere accidental features of preservation in accord with the distinctly granular character of the matrix.

WILLIAMSONIA ELOCATA Lesquereux. Fig. 5.—LESQUEREUX, LEO, Flora of the Dakota group. U.S. Geol. Survey Monograph XVII, pp. 87, 88. pl. II, figs. 9, 9a. 1891; SEWARD, A. C., Fossil plants, Vol. III, p. 462. 1917 (*Williamsonia elongata*; *elongata* merely a typographical error).

This singular problematic type from Ellsworth County, Kansas, has received no critical notice since its original description. SEWARD gives a bare synonymic mention of it as a fossil of indeterminate feature in his textbook, typographically as *W. elongata*. The original LESQUEREUX type was based on a single specimen, and no further material of interest has turned up until recently, as related in the description of a second form given later, under the caption of a new species. It is desirable accordingly to review LESQUEREUX's description. The *W. elocata* type was submitted by LESQUEREUX to SAPORTA for a critique, and by him referred to the Balanophoreae or "broom rapes." SAPORTA took a variant view of the Williamsonian types as unrelated to cycads, in which he was followed by

several other continental botanists. The type of the *W. elocata* has not been seen for many years. LESQUEREUX says in his description that SAPORTA retained the specimen.

Unfortunately the original drawing of *W. elocata* was reproduced as a halftone; however, the subjoined fig. 5, natural size,



FIGS. 5, 6.—Fig. 5, *Williamsonia elocata* Lesquereux, from Dakota (?) Cretaceous of Ellsworth County, Kansas; head or capitulum above, with imprint or fractured basal surface of such a head below; redrawn from LESQUEREUX; natural size. Fig. 6, *Williamsonia cretacea* Heer, from Atane beds (Atane-kerdluk) of Greenland; length of original 7 cm.; after HEER.

brings out the main features of the original drawing. The description given by SAPORTA as translated by LESQUEREUX follows:

We have recently received from our friend, LEO LESQUEREUX, another fossil organism, or rather the hollow mold of the organism, discovered in the ferruginous sandstone of the Dakota Group, therefore of the Cenomanian. One perceives in the specimen, after molding the cavity in relief, a thick, short receptacle shaped like an ovoidal, conical ball, mostly naked, and marked on its surface by scars of insertion, regularly placed in spiral, of a mass of scales, closely contiguous, inserted at right angles upon the receptacle and surrounded by a thick, spinous apophysis, subulate at base, shorter and less protruding toward the apex of the organism. These scales, which answer evidently to sexual elements, easily disengaged at maturity, are not without analogy, either by themselves or by the structure of the receptacle upon which they were



implanted, with the corresponding parts of the floral spadices of *Williamsonia*. If this analogy is real, we would have here a sessile, naturally caducous receptacle detached after the anthesis from an involucre of which it would have occupied the center. But here, without better evidence, it is difficult to pass above simple conjecture.

LESQUEREUX says of his fruit that it was round or reniform in outline, 4-5 cm. broad, and 3 cm. in vertical diameter; and then he adds an interesting detail unfortunately never illustrated. He states that the fruit was borne upon a cylindrical scaly pedicel or branch 1 cm. in diameter, the scales being 1 cm. long from their enlarged attachment. The cone scales are described as "closely imbricate, flat, heavy behind seeds or bearing pods which are falcate, 1.4 cm. long and 1.5 cm. in diameter, and transversely undulate at the surface as in some small seeds." It may develop, however, that this agrees quite closely with the fossil photographed in figs. 7-10. Faint transverse markings were noted on a seedlike body in one of the sporophylls, but pod cavities, with "small seeds," is an alternative explanation.

*Williamsonia* (?) *hespera*, sp. nov. Figs. 7-10.—The mold of a gymnosperm strobilus, or more likely "head" of some dicotyl, 5 cm. in diameter, with the stem which bears it, as shown in the photographic figures, may be a second example of the *W. elocata* of the preceding account. Differences of proportion, however, and perhaps of structure make the use of a new specific name a convenience if not a necessity. In any case this is one of the most curious of ancient fruits. The specimen, as already mentioned, was courteously sent to Yale for study from Washburn College, Kansas, in answer to inquiry concerning the silicified cycad trunk type known as *Cycadeoidea munita*, which has since been found and is a typical cycadeoid of uncommon interest.

No label giving the locality or other fact accompanied the specimen, but several trays full of the characteristic plants from the great dicotyl forests of the Dakota Cretaceous of Kansas, collected 50 years ago by CHARLES STERNBERG and sent to MARSH, afford a clue to horizon and locality.

These leaf specimens are characteristic perfoliate aralia and sassafras-like types; with one or two exceptions readily identifiable

from the figures of dicotyledonous foliage in LESQUEREUX's monograph of the flora of the Dakota group. The matrix is a soft, often yellowish nodular sandstone, precisely of the same character as that of the Washburn College fossil, so that the latter must be closely associated in some one of the Ellsworth County localities. It is to be added, however, that these Dakota dicotyls extend across several

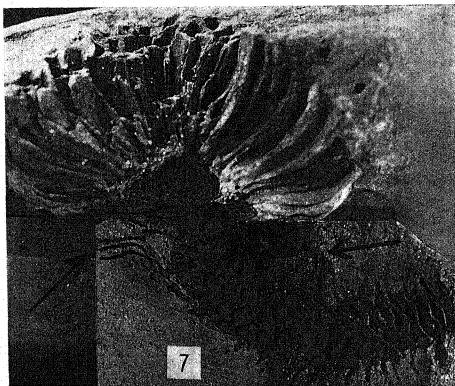
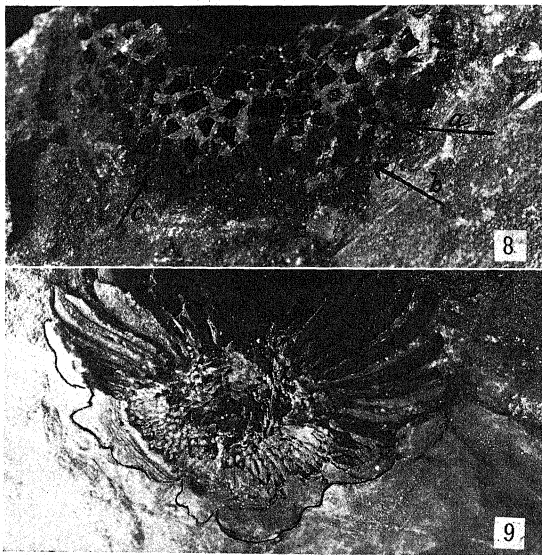


FIG. 7.—*Williamsonia* (?) *hespera*, type from collections of Washburn College; attached stem or peduncle brought into view by saw-cut through empty sandstone mold; cone or head broken open roughly along its diameter, and exactly in place with reference to stem. Note beneath the scale or sporophyll mass the receptacular cavity. Arrows show exact position and length of thin spinose leaves borne by stem or peduncle. Broader scars of bracts just at base of cone or head appear, but not the bracts in this view. (Cone width, 5 cm.; stem length 4 cm.; stem diameter, 8 mm.) See succeeding photographs.

counties, more or less northeasterly. The series in LESQUEREUX's monograph includes various related forms of leaves from the southwest corner of Cloud County (Glascoe), and the northwest corner of the intervening Scott County (Delphos).

In figs. 7-10 the main features of the new fossil may be visualized. An oblique shearing break through the fructification and across its

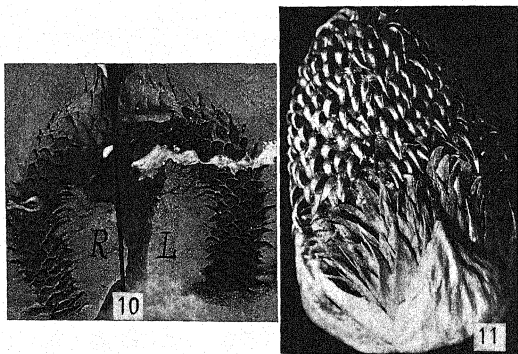
base discloses nearly all details, so far as conserved in the mold. In fig. 8 the fossil is seen from above, and in fig. 9 from below, the



FIGS. 8, 9.—*Williamsonia* (?) *hespera*: Fig. 8, photographed from above; note transversely fractured cavities left by disappearance of scales or carpels, or sporophylls. These transverse fractures in reality are serial from near insertion at center of receptacle (S), to broader portion 1.5 cm. out, and thence to bifurcate or paired, spined, or split summits. Note arrows (a, b, c,) pointing out paired spines of scales (if not ends of pods). This fossil is interpreted as a mold formed by imbedding of original capitulum in muddy fine to quicksands. (Diameter of head or cone 5 cm.). Fig. 9, strobilus or head photographed from below, showing broad bract insertions and flatly conical receptacular cavity. This view of base is at level of arrow (S) fig. 7. Compare with fig. 5; (diameter 5 cm.).

conical form of the receptacular portion being clearly outlined. Fig. 10 shows the two opposite sides of the attached stem or stalk,

as disclosed by a longitudinal saw-cut through the matrix. It was easy to locate the plane for this cut, as enough of the stem could be seen just below the cavity denoting the receptacular region, to indicate the direction taken by the curved stem through the matrix. The stem ends very abruptly in the matrix, about 4 cm. below the base of the fertile head, as if broken off transversely before fossilization. Even the outlines of the woody cylinder some 3 mm. in diame-



FIGS. 10, 11.—Fig. 10, *Williamsonia (?) hespera*: Lower Cretaceous of Kansas. Two opposite halves of cavity or mold of peduncle as brought into view by longitudinal saw-cut; scars of leaf spirals gradually elongate toward bract region; Washburn College specimen. (Stem length 4 cm.). Fig. 11, *Araucaria imbricata*: immature staminate cone from pure stand forests of Upper Bio Bio Valley, Chile; note that this is one of a close-set terminal cluster of five cones, and that following the larger stem leaves are more bractlike leaves basal to cone. Compare with fig. 2; (length 5 cm.).

ter are distinct at the end of the stem mold. Finally, on placing in position one-half of the stem resulting from the saw-cut, the photograph for fig. 7 was made. This comes very near to affording a complete picture, not only of all larger features of the peduncle and head or capitulum, but of much of the structure.

It is first noted that the spirally placed scars of the stem bear rather long leafy scales, or bracts as they must be called, at the strobilar base. These are rather broad and thin, the more distal

portions being somewhat obliterated on the face of the saw-cut. Their precise position and length, as borne along the stem in a close spiral series of nearly imbricant sigmoids, is never in doubt, however, and is indicated by the two arrows (fig. 7). The bracts normally broaden at the transition into sporophylls proper, as can be seen in fig. 9.

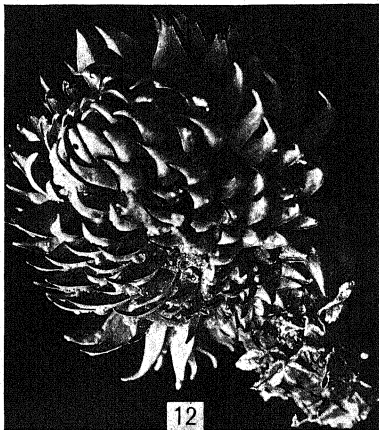


FIG. 12.—*Pinus coulteri*: aborted seed cone from San Bernardino Range, California; (diameter 5 cm.).

#### SPOROPHYLL SERIES

So far, the features might conform to some brachyphylloid or araucarian stem and cone, of Jurassic or Cretaceous age. Photographs showing young ovulate and staminate cones of *Araucaria imbricata* further illustrate the point. Also, the bract features are not inconsistent with the leaves of cone-bearing stems of other conifers, as in an aborted cone of the *Pinus coulteri* from the San Bernardino Range (fig. 12); in fact, the latter figure gives a certain rough approximation to the general size and form of the fossil fruit. It comes near to a restoration, if no attention be given beyond the

fact that the sporophylls (or other structure units) were of about the size and curvature, with diffuse spiral position, seen in the aborted pine cone.

Here, however, analogy ends and doubt begins. Around the bases of the sporophyll areas are small and long lateral slits or cavities, which might have corresponded to about three thin scale-like bodies about each sporophyll or unit. The number is not certain, although if these were infertile scales inclosing one-seeded sporophylls there would be a close approximation to a cycadeoid ovulate fruit. Likewise *Araucaria* would be approached, depending on whatever was the character of the seed; but here another difficulty arises. The sporophyll cavities or units may indeed represent a series of carpophylls, or even podlike fruits, and whatever was their nature they have the curious features of ending in two regularly oriented awl-shaped points about a centimeter in length. The division between these points begins well down in the sporophyll area, and always lies in a plane radial to the center of the cone. Where broken across near their tips *a-c* (fig. 8), the paired sections of these spiny ends are seen to be small and sub-triangular. These paired spines are not an araucarian feature of course, but they do recall somewhat vaguely the two-horned sporophyll of the cycad *Ceratozamia*.

The individual sporophyll areas, as they may be called for convenience, are present therefore as a series of larger cavities in the strictly spiral order. Each is flat to angular, elongate, and bifurcate, instead of "swollen" as in the *W. elocata*. About each larger cavity are the small scalelike cavities denoting unexplained organs. The greatest diameter of the serial cavities, about 3 mm., is reached at a distance of 1.5 cm. out, with bifurcation beyond that point into the two sharp, erect spines, a centimeter or more long. The "sporophylls" or units thus may or may not have been surrounded by, or have included scalelike anthers; and they may have been single seeded or many seeded dehiscent pods.

#### RELATIONSHIPS

It is difficult as yet to say whether this fruit is a later, highly aberrant form of one of the Cycadeoids, or actually the "head" of

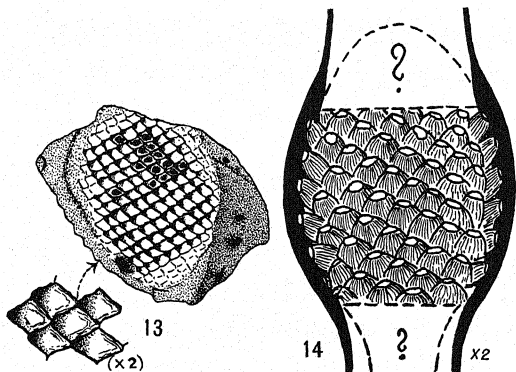
some early dicotyledon. A small body was noted in one instance resembling the cast of a seed about the size of a small grain of rye occupying most of the space, indicating the mold of the "sporophyll." It suggests the presence of a placenta and a series of pods forming a head of some leguminous plant. In a way this fructification is as difficult to understand as *Williamsonia* was originally. That structural riddle solved, this replaces it by one quite as difficult and perhaps as important. A certain relationship to cycadeoid cones depending on the final nature of the sporophylls and surrounding scales is only one of the possibilities. Yet hope of solution does not fail. Flowers found as casts or molds may also occur as impressions, and with both casts and impressions at hand, the anatomical characters might be discerned. The discovery of new material must be awaited.

#### GENERIC POSITION

That this type is specifically isolated appears certain, with the exception of the *Williamsonia elocata*. The stem features might agree with some of the various araucaroid or brachyphylloid stems abundant in Mesozoic rocks. But not knowing the family or even the order to which such a fossil may safely be ascribed, and with the hope that other examples may yet be found, there appears no immediate need to apply any other name to it than *Williamsonia*, a name well enough known to signify a whole tribe of extinct plants, as used from time to time.

The curious head, fig. 6, known as *Williamsonia cretacea* of Greenland, should also be recalled here. HEER describes this fossil at some length in the *Flora Fossilis Arctica* (Vol. VI. Mem. II. p. 59), also referring it to the Balanophoreae, as did SAPORTA and LESQUEREUX the *W. elocata*; and there seems little doubt that both forms are of the same genus, whatever the family to which they may pertain. It seems, however, to have been HEER and LESQUEREUX who referred such fossils to *Williamsonia* after NATHORST suggested that the group belonged to the broom rapes, a suggestion he soon emphatically abandoned. SAPORTA, however, thought the *Williamsonia elocata* should be given a new generic name. Through the fortunate conservation of the greatly specialized silicified cycadeoids, the structure of the cones definitely falling within the genus *William-*

*sonia* is now understood; but it does not seem that either the Greenland fossil or that just described need as yet be given new generic names in the absence of material eventually to be found. Paleobotanists and well informed botanists know that the fossil genera cannot always be given full precision, and are often highly inclusive, merely genera of convenience. Some fossils are definable within the narrowest limits; others scarcely within the family, the tribe, even the order.



FIGS. 13, 14.—Fig. 13, *Araucarites* (?) *polycarpa* Tenison-Woods from Lower Cretaceous of Urangan Point, Pialba, Queensland, Australia; for comparison with figs. 1, 2, etc. From Walkom; (diameter of cone 3.5+ cm.). Fig. 14, *Bernetia inopinata* Gothan. Rhaetic of Nürnberg: cone of unknown relationship, with Araucarian sculpturing, and large cycadeoid-like outer bract husk. From Gothan; (diameter about 3 cm.).

### Conclusion

The fossil plant problems must be kept continually in mind in both the field and the laboratory. Thus alone can progress be achieved in the study of ancient types. Among more recent fossil fruit discoveries of uncommon interest is a bract-enveloped cone from the Rhaetic of Nürnberg, assigned by GOTHAN to the new genus



and species *Bernettia inopinata*.<sup>3</sup> This fossil, fig. 14, is another addition to the forms promising determinate features in the evolution of the great groups. *Bernettia* is outwardly like some reduced araucaroid cone, with the final stem leaves developed into great bracts, recalling *Williamsonia*. GOTHAN places this cone under the Cycadophytes because of the bract features common in *Williamsonia*, with the outer cone features not unlike those of some modern cycad. Yet an araucaroid or even pinoid analogy may quite as well be hypothesized, according with the foliage of Cordaites, or of some one of the many Rhaetic gymnosperms.

GOTHAN also describes from the same region two cycadeoid-like cones, under the new generic names *Piroconites* and *Bennettitacearum*. The former is 5-6 cm. long and pear-shaped, the latter a slightly longer spindle-form fruit. Both have very small sporophylls, and, unlike the foregoing problems, may be assigned to the cycadeoids with some confidence. Even as indistinctly known from surface characters, these cones afford further evidence of abundance and diversity in the cycadeoids or Hemicycadales. Once this great group was known only from the casts and imprints first gathered by WILLIAMSON and his father just 100 years ago; now it is seen to be one of the most remarkable groups of ancient plants. That in the course of fossil plant investigation other Mesozoic groups of high importance in conceptions of the evolution of the flowering plants must come into view cannot be doubted. So many important facts have been learned even now, that the origin of the higher groups of plants is no longer a mystery, as DARWIN said in 1879. Probably botanists, because the fossil plant record is so frequently histologic, ask too severe proofs for origins.

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<sup>3</sup> GOTHAN, W., Die Unter-liassische (Rhaetische) Flora der Umgegend von Nürnberg. Ab. Naturh. Gesell. Nürnberg 4:91-186. pls. 17-39. 1914.

# CYTOLOGICAL STUDY OF THE INTRACELLULAR BODY CHARACTERISTIC OF HIPPEASTRUM MOSAIC<sup>1</sup>

FRANCIS O. HOLMES

(WITH PLATE III)

The intracellular bodies characteristic of corn mosaic, sugar cane mosaic, tobacco mosaic, *Hippeastrum* mosaic, and related mosaic diseases have been studied by a number of investigators. Some have suggested that the bodies represent living organisms. The general appearance of the bodies is that of vacuolated cytoplasm, and their close association with the affected areas of the host plants makes the hypothesis seem reasonable. The lack of any visible nucleus in the mass has been the most important argument against the supposition that the body represents some stage in a parasitic organism. In the absence of any substitute for this criterion, the opposing idea that the body represents a waste material or distorted cell constituent has gained a large following.

IWANOWSKI (7) in 1903 described the bodies in tobacco mosaic, giving an almost complete description of them as they are known today, but interpreting their granular structure as proof that they were masses of bacteria. KUNKEL (8) in 1921 described and pictured the inclusions characteristic of corn mosaic, enumerating the ways in which the bodies resemble amoeboid protozoa, and pointing out the lack of characteristic nuclei. He later published accounts (9, 10) of the similar intracellular inclusions in *Hippeastrum* mosaic, sugar cane mosaic, and the mosaic of Chinese cabbage and tobacco. GOLDSTEIN (2) and RAWLINS and JOHNSON (11) described the appearance of the inclusions in mosaic tobacco. SMITH (12) studied a number of infectious and non-infectious chloroses, and found that the vacuolate bodies occurred only in the infectious type. HOGGAN (6) recently examined many hosts of tobacco mosaic, and found intracellular bodies consistently in all hosts which showed characteristic macroscopic

<sup>1</sup> Contribution from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York.

symptoms. She found no such bodies in the same hosts when affected with cucumber mosaic. GOLDSTEIN (3, 4) made extensive studies of the intracellular bodies of tobacco and dahlia, and reported the presence of the bodies in dividing cells, and the division of the bodies there with subsequent distribution by the division of the host cells.

The object of the present paper is to set forth the results of the writer's study of the inclusion bodies which occur in *Hippeastrum* mosaic. The work has been done from the protozoological viewpoint, treating the structure provisionally as though it were a protozoan, the exact systematic position of which was in doubt. It has not been possible to prove whether the structure is or is not a parasitic organism. It has been possible, however, to carry out studies which furnish data not before available. A careful search for nuclei and nuclear material within the body, and an equally careful study of the chondriosome content of the body have been made.

### Materials

Almost all of the work herein described was done on a mosaic infected stock of the species *Hippeastrum equestre* (Herb.), which was available at the Institute. Since it proved to be impossible to secure seed from this stock because of clonal sterility, and since no healthy plants were at hand, it was necessary to carry out a part of the investigation on a stock of seedlings obtained from hybrid plants closely resembling this species. The seedlings raised in the greenhouse were invariably free of mosaic, whether from apparently healthy or from the most severely infected parents. They remained completely healthy so long as they were retained in the greenhouse. By placing a portion of the lot of plants in the Institute garden during the summer, with mosaic plants in the same row, enough transmission occurred to give an additional stock of diseased plants.

*Hippeastrum* was used as a source of mosaic intracellular bodies for this investigation because the bodies in this plant are very large and very numerous, resembling in a general way those of corn and sugar cane.

### Search for nucleus in intracellular body

Keeping in mind the hypothesis that the intracellular body might be a parasitic organism, the search for a nucleus or nuclear material

was carried on in every way which seemed likely to lead to success. It was recognized as a general principle that the greatest variety of fixing agents and cytological stains present practically the same picture so long as they act on the same structures; but it was thought worth while to test the typical groups of fixers and stains in the hope that if nuclear materials were present, they might be recognized more easily in the shades of differentiation resulting from the various processes.

The following typical fixing solutions were used: Schaudinn's fluid (a mercuric chloride-fixing agent); Flemming's weak solution (an osmic acid-fixing agent); Bouin's fluid (a formalin picric acid-fixing agent); Carnoy's fluid (an absolute alcohol mixture); and Regaud's fluid (a formalin potassium dichromate-fixing agent). It was felt that these represented the typical fixers needed to insure enough variation in appearance of any structures which might be present in the bodies. The stains used were as follows: iron haematoxylin, destained generally with iron alum; Giemsa's stain, unmodified, staining nuclear material ruby red; acid fuchsin, long stain, destained in tap water; Flemming's triple stain. It will not be necessary in this paper to describe the methods of preparation and use of the fixing agents. For formulas for all of them, and a description of the staining methods, LEE's *Microtometist's Vade Mecum* may be consulted.

In addition to the cytological methods indicated, photography with monochromatic blue light was used to study the general ground material of the body. This light was of wave length 448.1 millimicrons, and was derived from the spark between magnesium electrodes. Many of the blue light photographs, such as are shown in the accompanying plate, show the extremely granular appearance of the bodies. The substance of the intracellular body is frequently very conspicuously granular, even when viewed in unfixed, unstained material. These photographs also show the general contours of the bodies as seen in section. Most of the pictures represent bodies from epidermal cells where no chloroplasts are present. Thus only the cell walls, the cell nuclei, and the inclusion bodies appear. In cells below the epidermis the chloroplasts are often conspicuous, and may be in close contact with the inclusion.

The inclusion bodies react in the same way as the cytoplasm of a young cell does to all types of fixing solutions. This is surprising in view of the fact that they are within the cytoplasm of relatively old host cells. Fixing agents preserve more of their substance than is the case in the nearby fluid cell cytoplasm. Their contours are consequently more easily distinguished in stained preparations than are the contours of the enveloping cytoplasmic strands.

In the search for nuclear material in the inclusion bodies, only two types of structures were found which could possibly be interpreted as nuclei. In most lots of material from mosaic plants the bodies were found to possess a few granules capable of retaining stains intensely. When present, these granules were retained with all the fixing agents used. They stained intensely black with iron haematoxylin, purple with Flemming's triple stain, and brilliant red with acid fuchsin, as do protein granules in many plant cells. They were not observed in material stained with Giemsa's stain, but presumably took a blue stain if they were present, since they were not apparent against the uniform light blue of the intracellular body as stained by this method. Such granules as these are present in the cytoplasm of the *Hippeastrum* plant, and may perhaps represent stored food material. In the absence of any other characteristic than their ability to retain most dyes intensely, they cannot be said to resemble nuclear material very strikingly. Particularly is their absence from material stained with Giemsa's stain an argument against their nuclear nature, as will be mentioned presently. These brightly staining granules have been observed in the intracellular bodies of other mosaic diseases (8).

Giemsa's stain is capable of giving a very intense blackish red stain on nuclear chromatin, and a large series of sections of mosaic *Hippeastrum* was therefore prepared with this stain. The stain reacted perfectly in the immediate neighborhood of the intracellular body. Thus the host cell nucleus was so characteristically colored that even the smallest fragment of it was recognizable. The intracellular bodies were immediately adjacent to the well stained plant nuclei, but no nuclear material was shown in them. They stained uniformly light blue, like cytoplasm. It is believed that this Giemsa stain test is evidence that the bodies do not contain nuclear material.

The other nucleus-like structure within the inclusion body is one not previously described. It has the appearance of a sphere or spheroid, possessing one and occasionally two deeply staining balls of material located peripherally. Such spheres may occur singly, or there may be as many as a dozen within a single body. In diameter the spheres range from 0.75 to 2  $\mu$ . When present in numbers within a body they are not clumped, but well distributed through the mass. Besides being found in the intracellular bodies, these spheres are sometimes present in the cytoplasm of the host cell. In this location they are clumped in some cases, in others well distributed. They may be scattered between chloroplasts in cells containing such plastids.

Healthy seedlings have never shown such spheres in their cells, although search has been made in more than 50 plants. In plants grown in the field long enough to show slight traces of mosaic infection, these spheres have been found occasionally. In heavily diseased plants the spheres may be present in considerable numbers or entirely absent. Material fixed with Carnoy's fluid, Flemming's weak solution, and Regaud's fluid has been found to retain the structures. It happens that no material fixed with Bouin's fluid or Schaudinn's fluid has ever shown the spheres, but it is entirely possible that this is because too few lots were examined. With most dyes the spheres tend to take a surface stain, but the interior may sometimes be tinted. With iron haematoxylin and eosin the spheres may be outlined in black, or may be uniformly stained with eosin, the peripheral ball being deeply stained with the black of the iron haematoxylin in either case. With acid fuchsin, which stains ordinary nuclei brownish red and nucleoli brilliant red, the spheres are dull red and the peripheral balls brilliant red. With Flemming's triple stain the peripheral balls of intensely staining material become pink, the spheres being outlined in purple. With Giemsa's stain, which stains chromatin ruby red and nucleoli pale blue, the peripheral balls stain deep blue and the spheres stain in outline pale blue.

It is not certain what the nature of these structures is. Since they are the only conspicuous inclusion within the inclusion bodies themselves, an understanding of them may help to solve the ques-

tion of the nature of the intracellular bodies. Somewhat similar structures have been described in the cytoplasm of nerve cells in rabies by GOODPASTURE (5), and intranuclearly in human smallpox by CALKINS (1).

#### Chondriosome content of intracellular body

By a study of the chondriosome content of the body it was hoped that light could be thrown on some of the suggestions as to its nature. The numerous hypotheses advanced in the past to explain the intracellular body may be classified into four groups. (1) The body would be expected to have no chondriosomes in its mass if it were an abnormal chloroplast, leucoplast, chromoplast, or elaioplast, normal or abnormal nuclear material, a colony of virus particles free of host cytoplasm, or an abnormal tannin vesicle. (2) It would be expected to contain a moderate number of chondriosomes if it were a parasitic organism, living host cell cytoplasm containing virus, or living host cell cytoplasm without immediate contact with virus. (3) It might be expected to vary in chondriosome content if it were dying host cell cytoplasm or dead cytoplasm. (4) It might be expected to be completely composed of chondriosome-like material if it were a pile of chondriosomes aggregated, or fused, and perhaps chemically changing.

A typical lot of mosaic *Hippeastrum* tissue was fixed in Regaud's fluid, sectioned and stained with a long iron haematoxylin method. The chondriosome content of the cells and of the intracellular bodies was examined in a set of 100 slides.

The preparations consistently pointed to a definite conclusion. The intracellular bodies showed a moderate number of chondriosomes well distributed through their substance, just as the host cell cytoplasm nearby showed its expected quota, also well distributed. A group of photographs of a single intracellular body at different optical levels is shown in fig. 1, to demonstrate the uniformity of distribution of the chondriosomes in the mass of the body. The presence of a moderate number of well distributed chondriosomes in such a mass of material, in itself reacting like cytoplasm to all biological stains applied, is strong evidence for the view that the mass is

partly or wholly living cytoplasm, and is evidence against the opposing hypotheses which have been suggested. Of the possibilities, the three which are not opposed by this finding are: (1) that the body is a stage in the life cycle of a parasitic organism causing the disease; (2) that the body is a mass of host cell cytoplasm containing virus; or (3) that the body is a mass of host cell cytoplasm not immediately associated with virus, although perhaps holding its form because of the stimulation given by the diseased condition.

The hypotheses opposed by this finding of a normal number of well distributed chondriosomes within the intracellular body are also opposed individually by many small bits of evidence. Thus, for example, the probability that the body is not an abnormal elaioplast is indicated further by the fact that normal elaioplasts are found in the stems of mosaic and healthy plants alike, but no intergrading forms occur. Between the three possibilities not opposed by the chondriosome study no choice can be made at present, for no evidence is known to favor any one of them decisively.

### Summary

1. A cytological study of the intracellular bodies characteristic of *Hippeastrum* mosaic disclosed the fact that no nuclear material is to be found in the mass of the body, unless two types of structures should be so interpreted. These are (1) intensely staining dots not markedly different from others found outside the body in the fluid cytoplasm of the host cell; and (2) spheres containing deep-staining, peripheral, single or rarely double balls. These spheres are very definitely formed and easy to recognize. They were found also in the host cell cytoplasm in diseased plants, but not in that of healthy plants. These are the only formed structures of distinctive appearance within the intracellular bodies associated with *Hippeastrum* mosaic. It has not been possible to identify them.

2. Chondriosomes were found within the intracellular bodies in moderate numbers, well distributed through the mass. This observation is considered evidence for the view that the intracellular body in this particular disease consists of living cytoplasm. Whether the body represents a stage in a foreign organism, a mass of plant cell



cytoplasm containing virus, or a mass of the plant cell cytoplasm not immediately in contact with virus but stimulated by the diseased condition, is not known.

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#### EXPLANATION OF PLATE III

All photographs were taken with blue light from the magnesium arc, wave length 448.1 millimicrons.

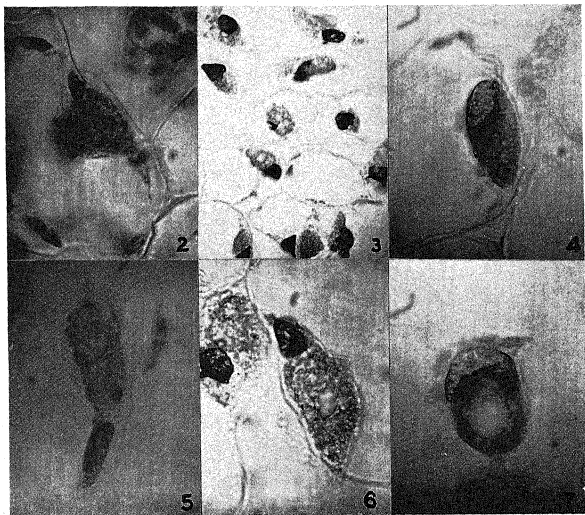
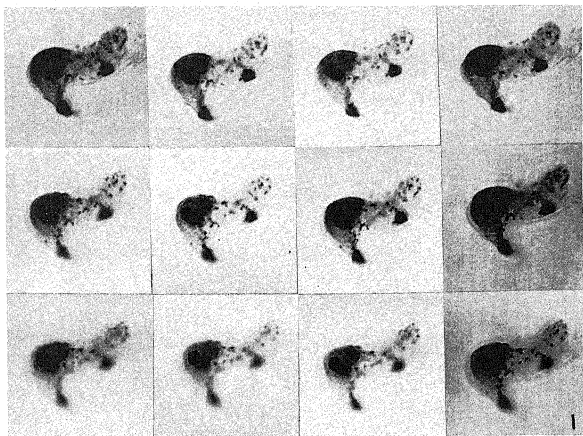
FIG. 1.—Nucleus and adjacent intracellular body photographed at twelve different optical levels to show that the dotlike chondriosomes are distributed

through mass of body and not merely on its surface; from mosaic *Hippeastrum* tissue fixed in Regaud's fluid and stained with iron haematoxylin; nucleus at left, intracellular body containing well distributed chondriosomes at right; two plastids below nucleus and body against wall of cell;  $\times 1000$ .

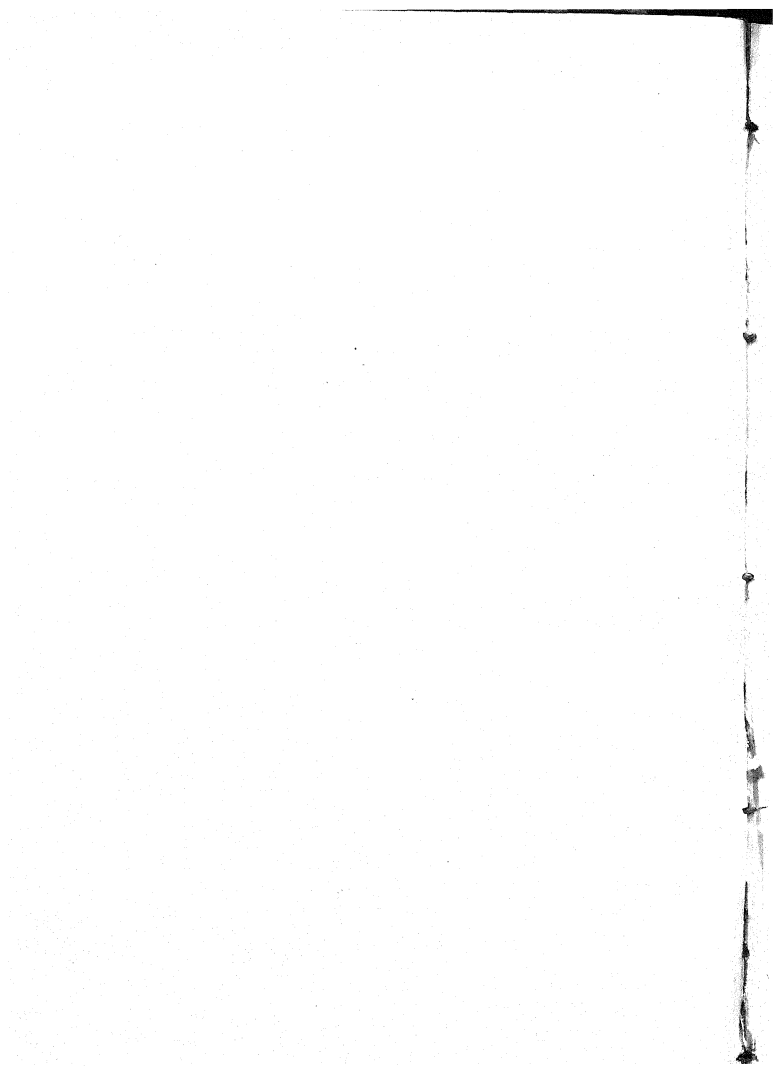
FIG. 2.—Intracellular body containing five spheres, only one showing in photograph; peripherally located deeply staining dot can be distinguished in sphere-like structure;  $\times 1000$ .

FIG. 3.—Low power photograph of section through epidermis of mosaic *Hippeastrum* plant; nuclei of cells appear very dark, bodies vacuolated and granular; fixed in Schaudinn's fluid, stained with Giemsa's stain;  $\times 300$ .

FIGS. 4-7.—Intracellular bodies showing granular structure, especially in fig. 6, vacuoles in figs. 6 and 7, close contact with nucleus in all; figs. 4, 5, and 7 from material stained with acid fuchsin following Bouin fixation; fig. 6 from material fixed in Schaudinn's fluid and stained with Giemsa's stain;  $\times 1000$ .



HOLMES on HIPPEASTRUM



## ULTRA-VIOLET LIGHT PHOTOGRAPHY IN THE STUDY OF PLANT VIRUSES<sup>1</sup>

FRANCIS O. HOLMES

(WITH PLATE IV AND ONE FIGURE)

It has seemed desirable for some time that photography with ultra-violet light, using a quartz-lens microscope, should be applied to the study of the infective juices of the virus diseases of plants. It was realized before the present work was undertaken that there were many difficulties which might make it impossible to obtain successful results. There existed the chance, however, that some one of the viruses available might consist of formed particles just too small to be seen with the visual microscope. If these particles were not too small and should possess a favorable refractive index, their images might appear on photographic plates exposed with light of short-wave length. The careful examination of these plates at leisure might make it possible to recognize structures characteristic of the virus-containing samples.

In order that this method of observation of the plants infected with a number of viruses might be thoroughly tested, arrangements were made with the Botany Department of Columbia University, through the kindness of Dr. R. A. HARPER, whereby their Zeiss ultra-violet light photomicrographic apparatus was made available to the Boyce Thompson Institute for Plant Research for a period of two years. It was hoped that a fairly complete preliminary survey of this field of investigation could be made in that length of time. The purpose of this paper is to report the methods used, the types of viruses studied by this means, and the results.

### Ultra-violet light photomicrographic apparatus

The apparatus used in the pursuit of these studies is shown in fig. 1. It was devised by KÖHLER, who described it thoroughly in

<sup>1</sup> Contribution from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York.

a long paper.<sup>2</sup> STEMPPELL<sup>3</sup> used this apparatus in his research which resulted in the photographing of the spiral filaments of *Nosema bombycis* within the spore. The apparatus is constructed to allow the sorting out of light of low-wave length from the cadmium spark, and the isolation of a relatively pure beam of light corresponding to the wave length of 275 millimicrons. This light is led through a

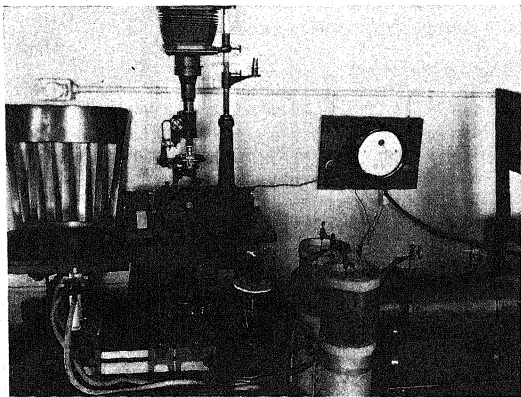


FIG. 1.—Zeiss apparatus for ultra-violet light photography: light obtained from a spark between cadmium or magnesium electrodes; quartz lenses and prisms isolate light of desired wave lengths and conduct it to microscope, which is equipped with quartz lenses.

series of quartz prisms, and passed through the microscope, which is provided with excellent quartz lenses adjusted for this exact wave length. Since the light is monochromatic, no chromatic aberration interferes with the sharpness of images secured. Objects to be photographed must be unstained and mounted between quartz slides and cover slips to insure complete transmission of the ultra-violet light.

<sup>2</sup> KÖHLER, A., Mikrophotographische Untersuchungen mit ultraviolettem Licht. Zeitsch. Wissen. Mikr. 21:148, 273. 1904.

<sup>3</sup> STEMPPELL, W., *Nosema bombycis*. Archiv für Protistenkunde 16:281-358. 1909.

The virus-containing juices used were quite satisfactory, in that they contained just enough debris to make it certain that focusing on the several layers of a given mount was accurate, and that suitable small objects within the range of the instrument were being depicted.

#### Typical viruses examined

The viruses to be studied were chosen with care to represent as varied types as possible. They were the following: aster yellows, tobacco mosaic, tobacco ring spot, potato witches' broom, potato leaf roll, potato rugose mosaic, potato aucuba mosaic. The aster yellows material was available at the Institute, where insect transmission experiments were under way. This virus causes chlorosis and the development of secondary buds, but no mottling or necrosis on the leaves. It is not mechanically transmissible. In complete contrast to this disease were tobacco mosaic and tobacco ring spot. The mosaic causes intense mottling with profound distortion of leaves, but with no necrosis; ring spot causes a necrotic pattern of concentric rings, but produces little chlorosis. Both of these tobacco viruses are easily transmitted mechanically, in contrast to the aster yellows virus which resists all attempts at transfer by rubbing or related methods.

On potato, additional types were made available through the kindness of E. S. SCHULTZ of the U.S. Department of Agriculture. The first of these was witches' broom, which is not related to aster yellows, but which causes growth of buds normally remaining dormant, thus simulating the appearance of aster yellows plants with less yellowing of the leaves. The second virus from potato was leaf roll, differing from all those thus far mentioned in causing no strong chlorotic or necrotic pattern on the leaf surfaces, but distinguished by a characteristic roll of the leaves. The third of this group was potato rugose mosaic, a mosaic comparable with the strong mosaic of tobacco in that it is readily transmitted mechanically. The fourth and last of the potato juices photographed was that containing the virus of aucuba mosaic, which causes isolated circular chlorotic spots on the foliage, but causes no noticeable leaf distortion or crinkling.

To collect a more varied group of viruses than these would be

difficult. It was necessary to have as representative a group as possible without introducing too many individual viruses, because the labor involved in properly studying a single additional type is considerable. It was hoped that these viruses might differ among themselves fundamentally, and that if size of the infective agent was the factor which has made it impossible to discover what viruses really are, some one of the seven might prove favorable in this respect, and furnish structures within the size range of the ultra-violet light instrument.

The juices to be photographed were obtained from the petioles of leaves of affected plants by pressing these against the quartz slide to secure a drop of fluid together with some débris from the broken cells. Such fluids in the case of tobacco mosaic are capable of causing the disease when introduced into healthy plants. The fluid was mounted for photographing between a quartz slide and a small quartz cover slip, and sealed in place with a ring of vaseline. The whole mount was placed at once under the microscope. A rough focus was secured with daylight by placing in the field of vision an air bubble purposely admitted into the preparation. This air bubble was still visible when ultra-violet light was passed through the preparation and caught on the fluorescent finder provided for the purpose. It could then be moved by means of the mechanical stage to a position at the edge of the field where it would appear in pictures taken at low magnifications, but not in the restricted fields taken by the more powerful quartz oculars.

Following the more careful adjustment of the focus by means of the fluorescent finder, a series of strip pictures was taken with a low power quartz ocular, the fine adjustment setting being recorded at each exposure. The plate thus secured was developed at once. With the best focus established, work began in earnest. The low power ocular was replaced by a higher. No inaccuracy was introduced in this way, as the lenses were made well enough to be in absolute agreement in giving a sharp focus. The same quartz objective was used throughout. A series of pictures was then taken, two on each plate, beginning with a point just below the correct focus and continuing to a point just above this focus. This allowed



a series of pictures to be examined for each spot of virus-containing juice photographed. It was found desirable to have at least a little débris from the plant tissues in each field to make possible an accurate comparison of the different levels, and to give assurance that delineation was satisfactory under the working conditions from day to day.

Difficulties were met in keeping the light intensity uniformly high, since a very slight change of direction of the beam causes a decrease in the illumination of part of the field at least. With light from the cadmium spark passing obliquely through a small eccentric stop and the highest objective, no decrease in intensity is permissible. Vibrations were eliminated as far as possible. The sealing of the plant juice in the mount and its subsequent exposure to ultra-violet light might seem to endanger the virus, yet motile bacteria handled similarly did not seem to be harmed, preparations after photographing appearing as fresh and as active as before.

It may be well to consider the conditions under which ultra-violet light photographs would be unable to depict small objects. If all the units to be studied were in active motion, whether showing Brownian movement or independent activity, no satisfactory images could be obtained. Among the most actively swarming bacteria, however, there are always some at rest on the surface of the cover slip, and the same is true of particles most of which may be in Brownian movement. If the refractive index were like that of the surrounding medium, and the absorption of ultra-violet light were low, particles would fail to impress their images on the photographic plate. The absorption of light by very small objects is in fact quite low. Small bacteria do not hold back much light, but because of their refractive indices they make distinct images on the photographic plate. The refractive index is sufficiently high in all known organisms to allow of photographing, but it might conceivably be low in the viruses. If this should be the case it might be impossible to see or photograph them. If, again, the causative agent were an organized structure smaller than 70 millimicrons in diameter, it would be hopeless to attempt to depict it with the type of short wave length apparatus now available. It would then be necessary

to have a powerful source of light of shorter wave length than 275 millimicrons to succeed.

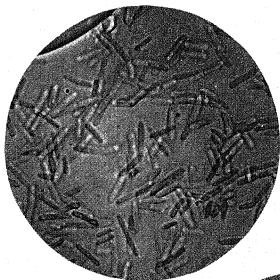
In this investigation the writer was concerned only with the range between the limit of resolution of the visual microscope, with particles in the neighborhood of 150 millimicrons in diameter, and the limit of the ultra-violet light instrument with oblique light with particles approximately 75-80 millimicrons in diameter. The results obtained from the whole study were negative. In the virus-containing juices of the seven diseases investigated, no formed particles were observed to differ from those depicted in the juices from corresponding healthy plants. A careful search was made through the extensive series of negatives obtained during two winters' work, but no structures characteristic of the infective juices could be detected.

About 600 photographs were made of the virus-containing juices. In addition a series of photographs of known bacterial plant pathogens was made for comparison. This series included *Bacterium tumefaciens*, *Bacillus amylovorus*, *B. caratovorius*, *Bact. campestre*, and *B. melonis*. The species were all photographed without staining, using living organisms. The results show that the ultra-violet light photomicrographic apparatus is of value in the study of the precise morphology of living, unstained bacterial organisms. It is already well known to be suited to the study of minute fungi and algae. KÖHLER and KRUIS<sup>4</sup> have published photographs of a number of species of living bacteria photographed in this way with ultra-violet light without the use of stains, but as the species were mostly unfamiliar, and KRUIS's publication is very difficult to secure, the five species of plant pathogens just mentioned are shown in an accompanying plate.

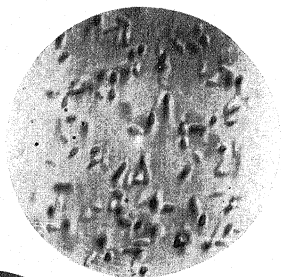
### Summary

1. Seven typical juices from plants affected with virus diseases were photographed with ultra-violet light of wave length 275 millimicrons, but no formed structures other than those seen in corresponding fluids from healthy plants were found.

<sup>4</sup> KRUIS, K., Rozprawy české akademie císaře Františka Josefa pro vědy, solvesnost a umění. Trída 2, R. 22:23. 1913. (Bacteria photographed with ultra-violet light.)



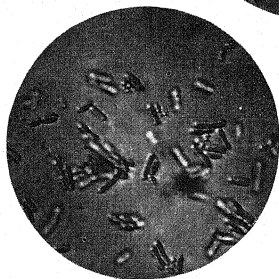
1



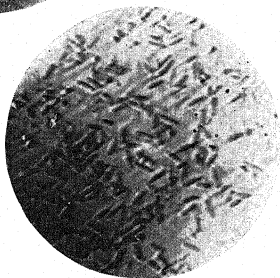
2



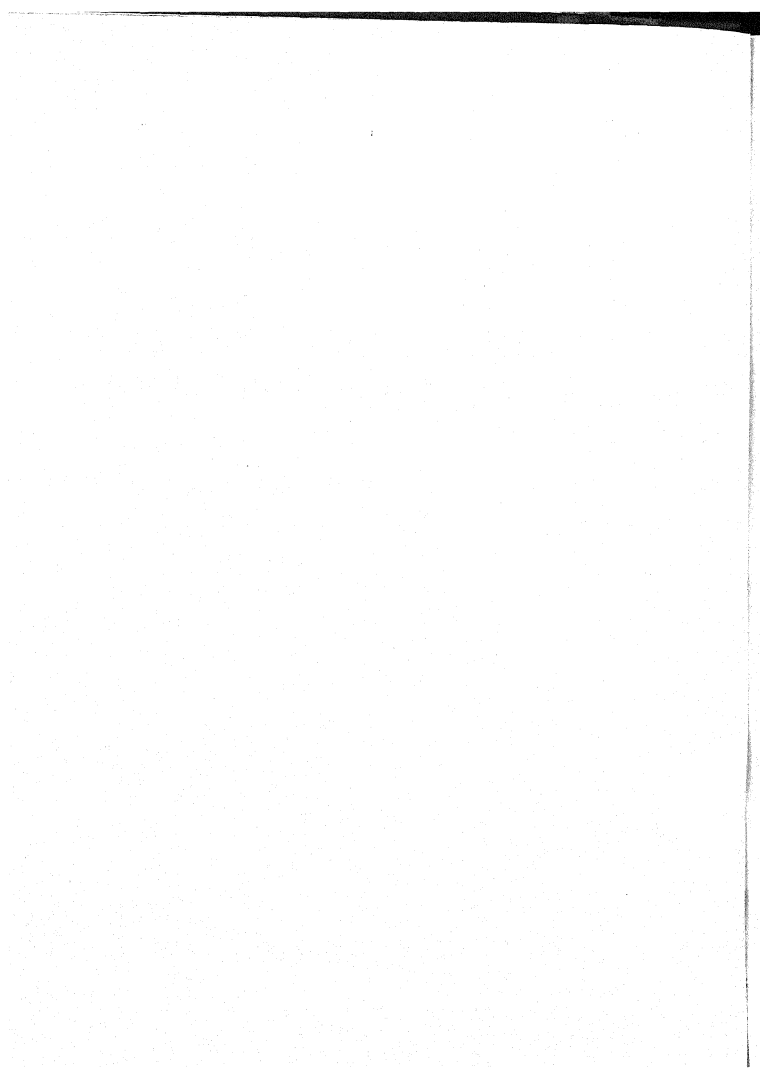
3



4



5



2. The plant juices photographed were from asters having aster yellows, tobacco infected with tobacco mosaic and tobacco ring spot, potatoes having witches' broom, leaf roll, rugose mosaic, and aucuba mosaic.

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#### EXPLANATION OF PLATE IV

All the figures are of unstained living bacteria,  $\times 2000$ .

FIG. 1.—*Bacillus caratovor*us.

FIG. 2.—*Bacillus amylovor*us.

FIG. 3.—*Bacillus melon*is.

FIG. 4.—*Bacterium tumefaciens*.

FIG. 5.—*Bacterium campe*stre.

## ACCURACY IN QUANTITATIVE WORK WITH TOBACCO MOSAIC VIRUS<sup>1</sup>

FRANCIS O. HOLMES

(WITH THREE FIGURES)

It is often desirable, in working with the readily transferred virus of tobacco mosaic, to have some means of telling whether one sample of the infective juice is more infectious or less so than another. This has frequently been accomplished by inoculating from ten to fifty plants in one of the accepted ways of mechanically transmitting the disease, and then judging from the number of resulting infections which of the two sources contained the larger proportion of virus in a given volume. The accuracy of this procedure is sufficient to allow a correct differentiation to be made between strong and weak samples of virus. Samples differing but slightly in strength can be differentiated and properly graded only by inoculating larger numbers of plants.

A recent paper by MCKINNEY<sup>2</sup> recommended a standard procedure for quantitative studies. He emphasized the difficulty of growing large enough numbers of plants in the greenhouse space usually available. The methods described in the present paper allow quantitative work to be done with economy of space, convenience, and rapidity of manipulation while inoculating, and a high degree of accuracy. It is particularly important to know what accuracy may be expected in experiments with viruses of known relative concentrations. With this information in hand the numbers of plants needed for proposed studies may be calculated.

During the study of plant disease viruses by means of ultra-violet light photography, it was desired to know how many units of virus, if such exist, were to be found in the small volumes of

<sup>1</sup> Contribution from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York.

<sup>2</sup> MCKINNEY, H. H., Quantitative and purification methods in virus studies. *Jour. Agric. Research* 35:13-38. 1927.

material represented in the individual photographs. For this reason the methods described in this paper were worked out. It was later found possible to obtain data from which the accuracy of such quantitative determinations of virus strength could be calculated, and from which the numbers of plants required to perform given inoculation experiments could be estimated in advance.

#### Procedure in making measurements

Since large numbers of inoculations were to be made, in order to reduce the effects of chance errors, it was evident that some uniform but very rapid method of performing the inoculations would

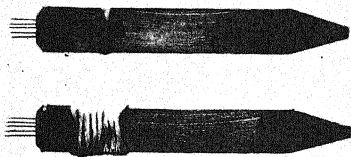


FIG. 1.—Inoculating needles, described in text

be needed. Punctures made with black enamel insect pins, size no. 00, were found to produce wounds of exceedingly regular size. Through these pin pricks tobacco mosaic was readily transferred, merely by alternate punctures in leaves containing virus and in leaves of healthy test plants. The pins used were obtained fresh for each experiment, so that no contaminations occurred because of the inoculating instruments. The fine points of the pins penetrate the leaves with the slightest pressure. It is not necessary to touch the plants at any time with fingers or instruments other than the insect pins actually used to introduce the inoculum. The uniformity of the dose lifted on the pins and transferred to new plants from mosaic specimens or from extracted juice is evident from the results. To obtain a fair percentage of infections it is necessary to make about five pin pricks in each test plant. The work of transfer

is therefore very greatly facilitated by using, instead of a single insect pin, a set of five pins bound in a temporary handle between two wooden pot labels (fig. 1). Rapidity of inoculation and uniformity of dose being thus combined, it is possible to make the required inoculations at the rate of 500 or more an hour. This method was found to be very satisfactory, and allowed many interesting experiments to be performed. Greenhouse space must be conserved if large numbers of determinations are to be made. The use of the simple inoculating apparatus just described makes it possible to utilize small tobacco plants. The wounds made by the pin prick inoculations into the leaf tissue are so small and heal so readily that even young plants are not held back by the introduction of the inoculum. No dead tissue appears around the site of the inoculation, as happens sometimes with rougher methods. It has been suggested that such dead tissue may hold back part of the virus applied and prevent its entry, so that the clean healing under the circumstances seems favorable.

In all of the experiments described in this paper, Turkish tobacco was used, because of the desirable shape of the plant for greenhouse studies, and because of its marked susceptibility to the mosaic disease. Its upright habit of growth and short leaves economize space. The tobacco plants were transferred to flats when they had a leaf spread of about one inch, and when well established were used for inoculation. The results can often be obtained before serious crowding begins, but crowding never caused trouble by the accidental transfer of the disease. Judicious care in handling plants and freedom from insects allow tobacco mosaic experiments to proceed with practically no contaminations.

In fig. 2 are shown the types of plant used. The wooden flat at the left contains small plants which have just been transplanted to it. The middle flat shows plants established and ready for inoculation. The flat at the right contains plants already diseased and ready to be discarded. So many plants can be placed in a greenhouse bench, when they are thus planted in wooden flats, that the space requirement for performing extensive experiments in a quantitative way is reduced to a minimum.



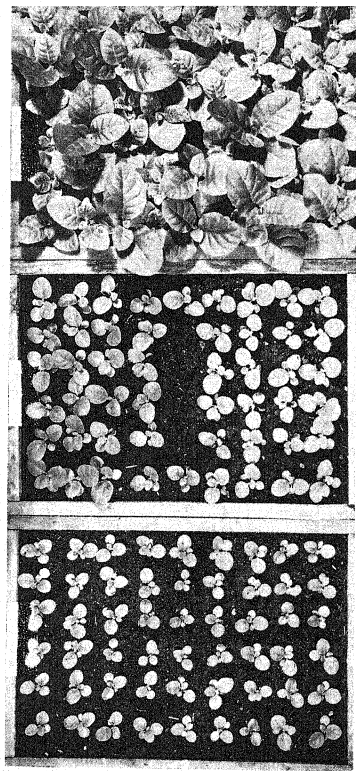


FIG. 2.—Turkish tobacco plants in wooden flats as used for quantitative measurements of tobacco mosaic virus strengths: at left, plants just transferred to flat; center, plants a week later when well established and ready for inoculation; at right, plants after results of inoculation are recorded, flat ready to be discarded.

### Results in measuring known samples

In order to know the errors involved in measuring the strengths of virus samples, experiments with definite strengths of virus were needed. A stock virus was therefore made by cutting 100 or more mosaic plants of Turkish tobacco into small pieces and expressing their juices into a glass bottle. The actual strength of this stock virus at any one moment was not known, but by measuring out one portion of this virus and mixing with it three portions of water, it is clear that a new sample of the virus is obtained, the total virus content of which, in a given volume, is one-fourth that of the original

TABLE I\*  
REDUCTION IN NUMBER OF INFECTIONS IN EACH SET OF 50 TEST PLANTS  
THROUGH USE OF KNOWN DILUTION

COMPARISON OF UNDILUTED VIRUS WITH	MEAN REDUCTION IN EACH SET OF 50 TEST PLANTS	P.E. SING.	NO. OF SETS COMPARED WITH UNDILUTED VIRUS	P.E. MEAN	STANDARD DEVIATION
1:2.....	3.7	4.5	20	1.0	6.5
1:4.....	7.5	4.6	24	0.9	6.7
1:8.....	11.2	3.3	16	0.8	4.8
1:16.....	15.3	5.2	16	1.3	7.4
1:64.....	22.4	2.2	8	0.8	3.1

\* For compactness in this table the mean reductions are given. As an example of the original figures obtained in the experiments, there follow the paired observations on undiluted and diluted samples from which the values shown in the 1:8 item above were calculated: 35-29, 29-8, 36-28, 28-12, 22-11, 11-4, 20-11, 11-3, 42-29, 29-19, 44-27, 27-21, 44-27, 27-20, 34-28, 28-11. It will be noted that the reduction on dilution is independent of the strength of the undiluted sample, as is implied by the graph shown for these values in Fig. 3.

sample. As will be shown later, this new sample upon inoculation into test plants does not give a reading one-fourth that given by the original sample, although it contains only one-fourth as much virus per unit volume. The effectiveness of a dilution for purposes of inoculation is not reduced by the addition of water so rapidly as is the actual concentration.

By adding water to the stock virus in other proportions, other definite dilutions may be obtained. A considerable number of such dilutions were prepared and all were promptly used for inoculation. Fifty inoculations require exactly five minutes. No great deterioration would be expected in such short intervals, and the consistency of the readings indicates that deterioration did not interfere with the process. In measuring known dilutions 2500 plants were used.

When the results were tabulated, as shown in table I, it was found that dilution to one-half original strength causes on the average a drop of  $3.7 \pm 1.0$  infections per set of 50 plants inoculated. This drop is independent of the strength of the original sample over the range studied. A dilution to one-fourth results in a decrease to the extent of  $7.5 \pm 0.9$ ; a dilution to one-eighth causes a decrease of  $11.2 \pm 0.8$ ; the still greater dilution to one-sixteenth results in lowering the reading by  $15.3 \pm 1.3$ ; and finally the dilution to one-sixty-fourth of the original strength causes a decrease in the number of infections amounting to  $22.4 \pm 0.8$  plants. A graph from which intermediate points may be read is shown in fig. 3.

As will be noted from the graph, the character of this response to dilution is such that the original strength of the virus does not affect the numerical reduction in infections resulting from the dilution. The numerical reduction due to dilution is dependent upon the degree of dilution, but not upon the strength of the sample taken for dilution. This makes it possible to use the information contained in the graph without the necessity for obtaining a virus of standard strength. With this information in hand, it is possible to calculate at any time the number of plants required to perform a given experiment in which it may be desired to differentiate between two viruses of moderately different strengths, or in which it may be necessary to compare two viruses with accuracy. If no limit to the number of plants available existed, it would be possible to obtain any desired degree of accuracy. Future improvements in technique may make it possible to determine virus concentrations as accurately as bacterial numbers may be estimated or chemical concentrations may be calculated from quantitative analyses.

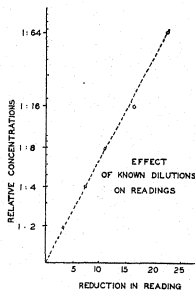


FIG. 3.—Graph showing effect of reducing virus strength by dilution; reduction in reading due to dilution is expressed in terms of average difference in number of infections resulting when undiluted and diluted samples are used, each in a set of 50 test plants.

For the present it seems necessary to be satisfied with a degree of accuracy demanding only a few hundred seedling plants. Table II shows the number of plants required to determine with good odds for significance the order of concentration of samples of virus of various strengths. Thus to distinguish with fair certainty between an undiluted sample and one a sixty-fourth as strong, some 30 plants are needed for each of the two samples in the comparison. If the difference between the two samples is but one to sixteen, 60 plants are required. If the difference is one to eight, 120 plants will suffice. If one to four, 240 plants must be used. If one to two, 1350 plants

TABLE II

NUMBERS OF PLANTS REQUIRED TO DETECT MODERATE DILUTIONS

$$N = \left[ \frac{\text{C.O.} \times \text{P.E. sing.}}{\text{Allowable deviation}} \right]^2$$

Average of P.E. sing. of difference between two dilutions = 4.0 (see table I)\*  
 C.O. for odds of 100:1 = 3.9      C.O.  $\times$  P.E. sing. = 3.9  $\times$  4.0 = 15.6

To compare undiluted virus with	Numbers required for each dilution; to be compared with same number from undiluted sample
1:2.....	$(15.6)^2 \div (3)^2 = 27$ sets or 1350 plants
1:4.....	$(15.6)^2 \div (7)^2 = 4.8$ sets or 240 plants
1:8.....	$(15.6)^2 \div (10)^2 = 2.4$ sets or 120 plants
1:16.....	$(15.6)^2 \div (14)^2 = 1.2$ sets or 60 plants
1:64.....	$(15.6)^2 \div (20)^2 = 0.6$ sets or 30 plants

\*The probable errors of single observations as listed in table I are so nearly equal, and have in themselves so large a probable error, that in calculating the numbers of plants required for experiments their average is used.

will be essential for the high degree of certainty implied by the odds of 100:1 that the difference observed is significant. It seems worth while to use these large numbers of plants since the labor involved is not great. The greenhouse space demanded is moderate compared with that often used in inaccurately determining virus strengths on smaller numbers of large plants. The equipment needed for the experiments is simple, being new insect pins for each experiment and a few wooden pot labels.

The formula by means of which table II was constructed may be of interest to some, since it is necessary in determining for any new experiment the numbers of test plants to be used. It is:

$$N = \left[ \frac{\text{C.O.} \times \text{P.E. sing.}}{\text{Limiting deviation}} \right]^2$$

In this formula  $N$  stands for the number

of plants to be used in the experiment contemplated; C.O. signifies the coefficient of odds corresponding to the degree of certainty desired (see PEARL and MINER<sup>3</sup> for complete table; 3.9 may be inserted here in the formula if odds of 100:1 for a significant result will be satisfactory); P.E. sing. stands for the probable error of a single comparison; limiting deviation is to be replaced in the formula by the figure representing the allowable error beyond which the set of readings must not go in the experiment to be performed, lest the significance of the results be impaired.

### Some applications of the method

#### EFFECT OF STORAGE ON SAMPLES OF PLANT JUICE CONTAINING VIRUS

It is evident that, since the error of experiments can be known accurately and adjusted within desired limits, many interesting questions can be answered, using enough plants in each case to obtain trustworthy results. Viruses from different sources may be graded accurately according to their initial concentrations, methods of preserving viruses may be compared as to their efficiency, and treatments with chemicals may be interpreted in the light of their exact effects. One very interesting and practical problem concerns ways of storing virus so as to have as slow a change in strength as possible. The practical importance of the matter comes from the need for some method of holding a given virus from season to season in order that experiments made at different times may be comparable. For example, it is desirable to know whether susceptibility of host plants differs with the change from summer to winter growing conditions, as some have suspected. This problem cannot fully be solved until some fixed reference point, such as a stable stock of virus, has been secured. It has long been known that tobacco mosaic virus allowed to stand in a bottle in the laboratory remains infectious for years. The question has never been answered, however, as to how rapidly the virus may lose strength at first. Most observers have noted diminished potency after long standing.

A rather extensive experiment was performed as follows. A

<sup>3</sup> PEARL, R., and MINER, J. R., A table for estimating the probable significance of statistical constants. Maine Agric. Exp. Sta. Bull. 226. 1914.

quantity of fresh virus, amounting to some 300 cc., was divided into two lots. One-half was stored in a glass bottle on the laboratory desk at approximately 22° C.; the other half was stored in a room kept below the freezing point. Before the latter sample was frozen it was poured into tubes. One-half of these tubes were allowed to stand frozen, the other half were melted and refrozen ten times. Each time the virus was poured from tube to tube in such a way that the clear ice of high melting point was gradually separated from the brown mother liquor of low melting point, and this in turn from the green sediment which contained all the solid débris.

#### TESTS WITH VIRUS KEPT AT ROOM TEMPERATURE

The first test of the fresh virus was made at once upon the collection of the juice. Five flats, each of 50 plants, were used for a series of small dilutions of this juice, the same dilution series being used throughout the course of this whole experiment, so that the total readings from the series could be compared each time with other totals. As soon as this set of five flats had been inoculated, a second series exactly duplicating it was arranged. Thus inoculations on 500 plants, contained in ten flats, were made with the fresh virus. As will be seen in table III, which records the results of this experiment, the first series of five flats used for this fresh virus gave a total of 117 infections. The duplicate series gave 140. The average number of infections per set of 50 plants over the whole series was therefore 25.7 plants.

When the virus had stood at laboratory temperature for 24 hours a second similarly arranged test was made. Five hundred plants were again inoculated in exactly the same way, but with the one-day old virus. It was found that the number of infections had greatly changed, and that the two duplicate series agreed throughout in giving evidence of a decrease in strength. In five sets 50 infections had appeared. In the duplicate five sets 51 infections were found. The average number of infections per set of 50 plants was 10.1 plants, a much smaller result than was recorded for the fresh virus. If the fresh virus concentration be taken as 100 per cent, then it will be noted by reference to fig. 3, which represents the decrease due to dilution, that this one-day old virus acted as a 6

per cent suspension would be expected to do. If the reader desires to inquire into the accuracy of the duplicate series it will be possible to do so, using 4.0 for the probable error of a single comparison as shown in table II.

Before considering the behavior of this stored virus sample upon further aging, it must be mentioned that this experiment when repeated gives changed results according to the bacterial flora present and developing in the sample, and that this particular

TABLE III  
EFFECT OF AGING ON EXTRACTED VIRUS AT 22° C.\*

VIRUS	DUPLICATE DETERMINATIONS	OBSERVATIONS ON SERIES OF DILUTIONS					TOTALS	AVERAGES	STRENGTH INTERPRETED IN TERMS OF ORIGINAL (PERCENTAGE)
		Undiluted	1:4	1:8	1:16	1:64			
Fresh.....	1....	35	29	29	16	8	117	25.7	100
	2....	36	36	28	28	12	140		
One day old.....	1....	22	11	11	2	4	50	10.1	6
	2....	20	9	11	8	3	51		
One week old.....	1....	42	41	29	35	19	166	33.0	400
	2....	44	36	27	36	21	164		
Two weeks old....	1....	44	43	27	22	20	156	29.7	210
	2....	34	38	28	30	11	141		
One month old....	1....	11	6	9	3	2	31	6.2	2
	2....	11	7	5	3	6	31		

\* The individual readings were made on a set of 50 test plants each time.

case is being recorded to indicate the actual findings in one instance. It is on the whole a good illustration of what happens to a virus at room temperature. Following the inoculations at the end of the first day a bacterial fermentation took place in the virus sample. Bubbles of gas rose through the liquid, and an odor like that of a fresh hay infusion was noted. This fermentation may have broken up solid particles in the suspension, thus liberating virus. Whatever its mode of action, a change in virus concentration was very evident when inoculations were again made at the close of a week. The first five sets of 50 plants, each inoculated in this seven-day sample, gave altogether 166 infections. The accompanying series

of five additional sets gave 164 infections. The samples for the duplicate series were all prepared independently, no one being made from another, but all from the undiluted stock bottle by the process of pipetting out known quantities of the virus and of water. The average number of infections per set for this one week test was 33.0 plants. This corresponds to the reading which would have been given by a sample four times as strong as the original. At the end of another week, fourteen days from the time when the fresh virus was tested, a new examination was made of the state of preservation of the virus, and it was found that two readings, 156 and 141, corresponding to the totals given before, were obtained. The average number of infections per set of 50 plants was therefore 29.7 plants. This indicated a strength still double that of the original, but lower than had been recorded at the end of the first week.

At the end of one month of storage the sample was again tested in the same way. The first 250 test plants gave 31 takes, the second 21 takes. It appeared then that a rather low level had been reached, the average number of infections per set of 50 plants being 5.2, and the strength of virus indicated being approximately 2 per cent of the original concentration. A subsequent test after four months indicated that a slow decrease had continued to take place. It must be remembered that this loss of 98 per cent of the strength after some weeks of standing does not mean that the virus would be considered very weak under some conditions, for when heavy inoculations are made as a regular practice, it is customary to dilute fresh viruses with water to such an extent that much weaker suspensions than this are obtained. It is possible to have 100 per cent of infections result from the use of these weak samples, because a large quantity of the virus is applied to the cut surfaces of the leaves. In many experiments, however, the loss of a large part of the virus strength is to be avoided, and in such work the results of this experiment indicate that speed of operation is a desirable feature.

#### TESTS WITH FROZEN VIRUS

At the end of a month and a half the sample of the original virus which had been frozen was melted and tested. It appeared that it had not lost as much of its strength as had the room temperature



sample. It had lost approximately 85 per cent of its effectiveness, however, as judged by comparison with the dilution chart. In the test which determined this, the average number of infections per set of 50 plants throughout the same series of dilutions as was reported in table III for the room temperature sample was 15.2, a reading lower than that given in the earliest test for the fresh virus by 10.5 plants. It is this difference which indicates the amount of loss due to the long standing while frozen. It will be seen, therefore, that the virus stored at room temperature was reduced to 2 per cent of its original strength in a month; that which was stored frozen retained 15 per cent of its strength as long as one and a half months.

When tests were made on the frozen fractions, which had been secured by thawing and pouring in such a way that one appeared to be clear ice water, one a deep brown low melting-point solution, and the third a mass of green *débris* suspended in a little fluid, it was found that the bulky ice fraction and the smaller *débris*-containing fraction had lost very little more of their strength than had been lost by the whole virus. At the same time the brown fraction had increased significantly in strength. Apparently the virus had been frozen out of the clear ice to a slight extent, and had followed the pigments and dissolved salts into the small volume of the low melting point fraction. Further work will be necessary before an unchanging virus can be secured by any method of storage. These experiments, illustrating the extent of the changes which may take place when a virus stands in a glass receptacle for some weeks, at least indicate that a virus does not retain all of its strength over long periods of time.

#### GREEN AND YELLOW AREAS OF MOTTLED LEAVES

It is known that mottled leaves contain virus in the yellow areas and also in their apparently green areas. A careful examination of the green areas shows that they are sometimes invaded by yellowish patches, and that mistakes may arise in taking them all to be representative of the purest green areas available on green and yellow leaves. Preliminary tests showed that by loading the insect pin inoculating instruments with juice by sticking them

directly into tissues to be investigated and then into the leaves of healthy test plants, a great difference could be observed in yellow and green areas respectively as sources of virus. While the experiment recorded below does not show that the green areas are free from virus, it does show with certainty that the green areas are poor sources of virus as compared with the yellow areas immediately adjacent to them. In each of the ten parts of the experiment here described a single well marked leaf was taken, and examined first

TABLE IV  
GREEN VS. YELLOW AREAS OF SINGLE MOTTLED LEAVES AS  
VIRUS SOURCES

LEAF NO.	YELLOW AREA	GREEN AREA	DIFFERENCE IN READING	DEVIATION FROM MEAN	DEVIATION SQUARED
1.....	30	11	19	1.2	1.4
2.....	16	6	10	7.8	61.5
3.....	21	0	21	3.2	10.0
4.....	33	2	31	13.2	174.0
5.....	28	6	22	4.2	17.6
6.....	21	1	20	2.2	4.8
7.....	29	5	24	6.2	38.4
8.....	12	2	10	7.8	60.8
9.....	12	1	11	6.8	46.2
10.....	12	2	10	7.8	60.8
			Mean=17.8	Sum=476	

$$\sigma = \frac{476}{10} = 6.9$$

$$Z = \frac{17.8}{6.9} = 2.58$$

$n=10$  Each reading was made on  
50 test plants.

Odds for significance 10,000:1; odds that yellow area is at least eight times as strong a source of virus as green area about 100:1.

by making transfers to 50 plants from the green areas, then by making similar transfers from yellow areas to an equal number of plants. The leaves were not chosen as comparable, but merely as having well marked patterns. The yellow area readings are not all of the same order of magnitude, as will be noted in table IV, where they are summarized, but the difference between the yellow area on a leaf and the green area on the same leaf is consistently large, and always in favor of the thin yellow tissue as the better source of infective material.

The number of infections from the yellow areas was 17.8 higher on the average per set of 50 test plants than the number of infections

from the green areas. By reference to the dilution chart it will be noted that this corresponds to the difference as virus source between an undiluted and a 1:28 virus. The odds that the average difference of 17.8 represents a real difference between the two sources are very high, approximately 10,000:1. It appears, therefore, that *in mottled leaves* the virus distribution is indicated by the yellow pattern. It is known that leaves below the mottled ones on a plant are not free of virus after the plant has been infected for a long time, even though they are green. They may contain virus in almost as great a concentration as that in the yellow portions of mottled leaves, yet some may be almost free of virus, depending on their previous history and their position on the plant, as will be seen from the following account.

#### SPREAD OF VIRUS IN INOCULATED PLANTS

By inoculating a series of similar plants near their growing points, and then examining their leaves from time to time by making large numbers of transfers to healthy test plants, it was possible to assemble the data summarized in table V. This table shows that the green leaves above the point of inoculation quickly built up a concentration of virus comparable with that found in the yellow areas of mottled leaves, but that the green leaves below the point of inoculation remained for many days free of virus, later possessing a considerable amount. From the time of the appearance of the earliest symptoms, it is evident from the large numbers of infections secured in surveying leaves above the point of inoculation, that virus was present in the upper part of the plant. Only the column marked "developing leaf" has to do with leaves which show clearing of veins or mottling. The inoculated leaves never showed symptoms, yet they came to contain large amounts of virus. It seems necessary to assume from these figures that the larger part of the virus present in these green leaves below the mottled ones, but above the point of inoculation, was formed in them, and was not due to a backward flow from the developing leaves, because the original point of inoculation marked the limit of the virus-containing leaves. If the virus should flow back down the plant in quantity, it would not be expected to stop at the inoculated leaf. Yet virus was found, from the

time of the appearance of the earliest symptoms until the end of three weeks from the time of inoculation, to be present in large amounts in the inoculated leaf and those above it, and to be absent from the leaves immediately below. The later development of virus in the lower leaves must indicate a migration of at least a small amount of virus backward down the plant.

TABLE V  
SURVEY FOR SPREAD OF VIRUS\*

	LOWEST GREEN LEAF	INTERMEDI- ATE LEAF (BELOW)	ORIGINALLY INOCULATED LEAF	INTERMEDI- ATE LEAF (ABOVE)	DEVELOPING LEAF
Inoculated when 8 inches high					
Examined at:					
Clearing of veins.....	0	0	29	43	42
First mottling.....	1	0	25	45	39
Three weeks.....	0	0	32	37	36
Four weeks.....	49	47	42	46	5
Five weeks.....	10	29	40	42	40
Inoculated when 1 inch high					
One week.....	.....	.....	11	30	24
Two weeks.....	.....	.....	28	38	42
Three weeks.....	.....	.....	35	48	43
Four weeks.....	.....	.....	45	39	43
Five weeks.....	.....	.....	33	44	44

\* Each number in the table here given represents the results of the inoculation of 50 test plants. The exact reading should not be considered important, since there is some variation when as few as 50 plants are used in each test. The groups of figures are consistent enough, however, to show the trend of virus concentration in the leaves in each position considered.

It seems necessary to conclude that the virus may develop and come to high concentration in leaves which appear very different, some being green, others mottled; and that mottling is not so much an indicator of the presence of virus as it is an indication that virus was present at the time the leaf developed. More careful studies will be made later. This survey indicates in a general way the behavior of the virus as it spreads through the plant. In addition to the points of theoretical interest involved, the survey throws some light on the length of time plants should stand infected before their leaves are used as sources of virus. It is hoped that the account here given of the method used for obtaining concentration readings on tobacco mosaic virus may be of value to other investigators who

would like to know the strengths of the viruses with which they may work, and what degree of reliance may be placed on readings of such strengths.

### Summary

1. A method of inoculating test plants with small, uniform doses of tobacco mosaic virus is described. The object of the method is to lessen the mechanical difficulties usually met with in the attempt to obtain reliable quantitative results. By the use of small plants, inoculated by pricks of insect pins held in a convenient handle, very large numbers of test inoculations may be performed with economy of time and effort, and with a minimum of greenhouse space and practically no danger of contamination from handling.

2. It is shown that dilution to a given extent causes a decrease in infections when inoculations are made into test plants, and that this decrease is not dependent upon the original concentration of the virus, but only upon the percentage dilution in the range studied. Charts and a graph are given so that the results with known dilutions may be available for grading unknown samples of virus. It is shown that the numbers of plants required for significant results in proposed experiments may be predicted.

3. Experiments are described showing that by the use of such methods it can be demonstrated that virus tends to die off rather rapidly in storage at 22° C., more slowly but still considerably when frozen; that the yellow areas of mottled leaves are much better sources of virus than the adjacent green areas of the same leaves; that green leaves above the point of inoculation may quickly become strong sources of virus, yet apparently similar green leaves immediately below the point of inoculation remain free of virus for some weeks, later becoming effective sources of virus.

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## FURTHER OBSERVATIONS ON STRUCTURAL DEFECTS OF THE GRAFT UNION

E. L. PROEBSTING

(WITH SIX FIGURES)

A preliminary survey of the structure of the graft union has been made, designed to give an indication of the types of unions to be expected, and some idea of whether or not correlation between field behavior and structure could be observed. Since the California Agricultural Experiment Station is engaged in extensive field trials of grafted material, a considerable number of combinations was available for study, in addition to specimens of various kinds and different ages from other sources. The Experiment Station material was largely one-year-old budded nursery stock. From one to six unions of each of thirty-one combinations were sectioned.

A brief description of a type of abnormality, consisting essentially of a double layer of bark accompanied by a considerable amount of wood parenchyma between stock and scion, was published in 1926.<sup>2</sup> The studies of which those observations formed a part have been extended to other interspecies grafts of *Pyrus*, and to several combinations of *Prunus* species.

Exceedingly great variations occur in the structural details at the line of union of stock and scion. The range may be said to extend from the combination which forms a perfect union, and which exhibits no defects of any sort, to the extremely uncongenial pair which does not form a union of any sort, and which results in the death of the scion. Just short of this latter extreme is the type just mentioned, of bark formation between stock and scion in which the scion may start growth, but in some cases may not live through the season. This type of union is found in some combinations of *Prunus* as well as in *Pyrus*, notably the Beauty plum (*Prunus salicina*) on almond (*P. communis*), and in the Italian prune (*P. domestica*) on the sweet cherry (*P. avium*). The former combination is illustrated

<sup>2</sup> PROEBSTING, E. L., Structural weaknesses in interspecific grafts of *Pyrus*. BOT. GAZ. 82:336-338. 1926.

in fig. 1. In this example there is a segment of the union that is nearly normal.

The normal union may be considered to approach the type shown by prune (*P. domestica*) on Myrobalan (*P. cerasifera*). In transverse sections of budded material the wood of the stock next the cambium is ordinarily summer wood. When the bud begins growing spring wood is the first laid down. The effect produced, therefore, is essentially similar to that produced by the adjacent summer and spring wood of two annual rings. The medullary rays are continuous across the line of union. The pits in adjacent cells meet as though the cells were derived from a single source. The cambium cells of the scion have formed medullary ray cells if they were in contact with ray cells, and xylem elements if they were in contact with xylem elements. It seems a curious circumstance that the cells of the stock should exert this influence on the differentiation of cells of the scion. This condition was found in all unions which did not exhibit some other type of structural defect, and in some unions which did.

The line of union in a transverse section of a normal union can usually be identified by differences in character of tissue or cell structure. Gross differences in the appearance of the two tissues, which can be seen without magnification, may sometimes serve as a means of orientation. It is often practically impossible to identify the last cell of one sort and the first of the other in sectioned material, even when the general region of the union is well defined.

In radial sections of budded material, the line of union is usually indicated by a sharp angle between the axis of the stock and that of the scion at the point of union. The bud (scion) having been inserted on the side of the stock, its first growth is in the same direc-



FIG. 1.—Beauty plum on almond, showing layers of bark extending part way across union; center of union sound.

tion as that which a lateral bud would make. After that part of the stock above the bud has been cut off, and the scion has grown over the cut surface, the growth rings in a normal union tend to form continuous cylinders, with the line of union becoming nearly transverse. This is shown diagrammatically by WAUGH.<sup>2</sup> With grafted material, the surface of contact between the wood of the stock and of the scion which had differentiated before the grafting took place, and which never unite, can be used as a means of orientation. Radial sections show the continuity of rays across the line of union as well as do transverse sections. Fig. 2 is an example.

The tangential section, especially in older specimens where the line of union has become nearly transverse in the younger wood, is usually the most striking, and shows abnormalities in the clearest manner. Differences in the character of the rays, vessels, or fibers are shown very well. In good unions the continuity of tissue is again shown by the way in which the xylem elements are dovetailed together to make a well knit, mechanically strong juncture. A section of this sort is shown in fig. 3. In poor unions the defects stand out sharply, as will be shown later.

Perhaps the most common type of structural defect is that involving the deposition of wood parenchyma by the cambium at the line of union, interrupting the vascular connection to a considerable extent. This may be seen in any plane. There is a great deal of variability in the extent to which this condition may be expressed. In some cases there may be only isolated masses of parenchyma with the vessels making fairly good connections around them. In others the ends of the vessels may be almost entirely separated by these cells. In extreme cases the vascular tissue adjacent to the parenchyma may show distortion, and the layer of parenchyma be continuous. It appears that the cambium of the stock, of the scion, or of both, adjacent to the line of union produces only parenchyma cells, giving practically a continuous sheet of these cells between stock and scion. The result of such a condition is a mechanically weak structure which may survive several seasons, but which will ultimately break off as the increased leaf surface of the top offers more

<sup>2</sup> WAUGH, F. A., The graft union. Mass. (Hatch) Agric. Exp. Sta. Tech. Bull. 2. 1904.



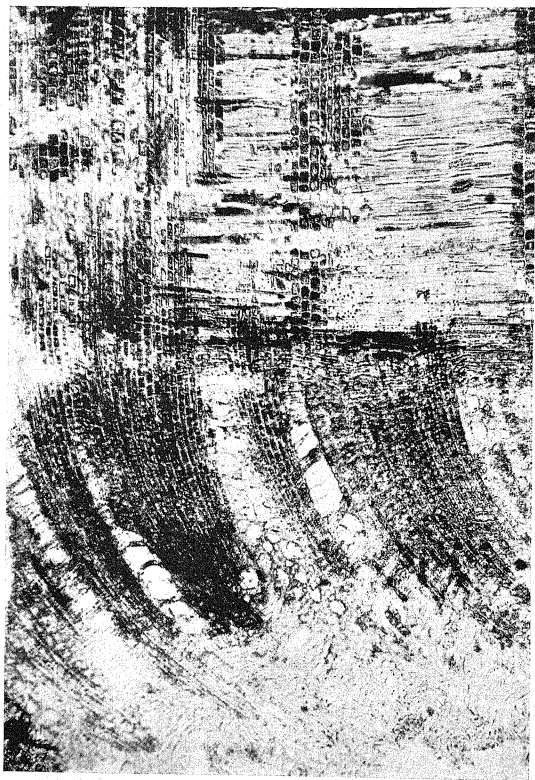


FIG. 2.—Radial section of union of Formosa plum on Myrobalan, showing change of plane of scion and continuity of rays across union.

resistance to the wind. Fig. 4 shows such a union which has broken off. This condition occurs in such unions as apricot on Myrobalan, which often breaks off in this manner, and in many other combina-

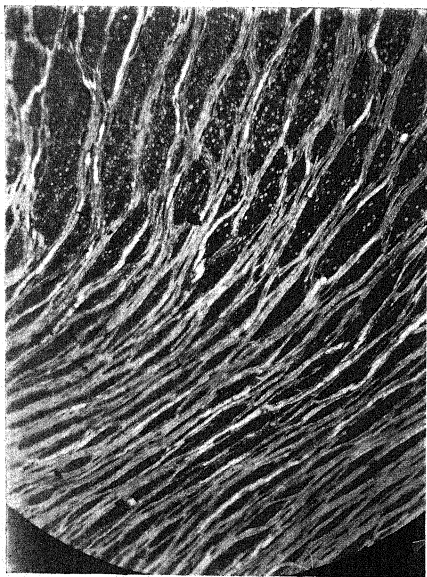


FIG. 3.—Tangential section of union of French prune on Muir peach, showing dovetailing of vascular elements of stock and scion; difference in size of rays identifies the two tissues.

tions in a less marked degree. This is shown in tangential section in fig. 5.

Distortion of the vascular tissue, while it may not cause either mechanical weakness or functional disturbance in mild cases, is commonly encountered in the neighborhood of the union. This distor-

tion may be confined to the formation of a sharp angle of xylem and of phloem elements, in the case of a stock which grows much more rapidly or more slowly than the scion. Almond on peach frequently shows this sharp angle at the line of union. It can be seen by inspection with the naked eye, as well as by the examination of sections.



FIG. 4.—Result of parenchyma formation at line of union of apricot on Myrobalan.

It has no apparent influence on the life of the tree or its behavior. BRADFORD and SITTON<sup>3</sup> have emphasized this point with regard to the apple. However, when the distortion reaches the degree of forming whorls and loops, and of showing vessels in both longitudinal and transverse section within the space of a millimeter, and when

<sup>3</sup> BRADFORD, F. C., and SITTON, B. G., Some cases of false uncongeniality in graft unions. *Proc. Amer. Soc. Hort. Sci.* 24: 1927 (in press).

the continuity of the vascular system appears to be definitely broken by these contortions, it is difficult to believe that there is not a functional disturbance as a result. Keeping in mind the danger of drawing conclusions bearing on function merely from observations on

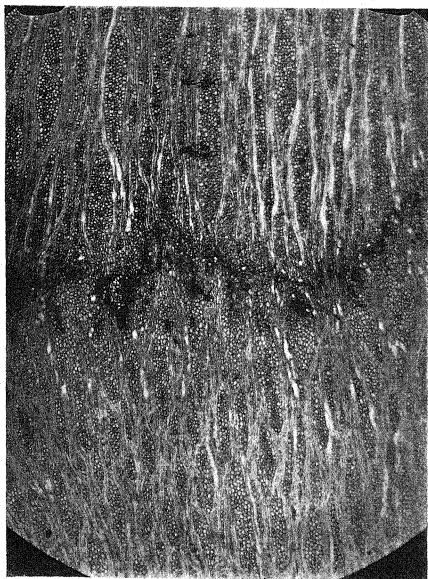


FIG. 5.—Tangential section of union of Phillips peach on apricot, showing wood parenchyma interrupting vascular continuity.

structure, and the fact that distortion is a normal feature of such structures as the nodal plates of corn, it should be recognized that some other factor may be of greater importance in governing the

behavior of such combinations in the field. These seriously distorted regions are sometimes associated with masses of parenchyma, and at times with the degeneration of certain areas to amorphous, gummy masses. The occurrence of such a condition is illustrated in fig. 6.

Another type of structural defect is found in some combinations of *Prunus*. In this type all of the xylem between the medullary rays at the line of union is degenerated into a gummy mass. These gum pockets are continuous around the medullary rays and may extend for considerable distances. Obviously, unless the region of gumming is confined to a portion of the xylem at the union, water movement would be expected to be very materially checked. If the area affected is small in comparison with the total surface of the union its effect would be small. The condition described may occur at the line of union only by chance, and not be an effect of the interaction or lack of interaction of the grafts. A very similar condition may occasionally be seen in limited areas in other locations than at the line of union. It is possible, therefore, that the gum formation is a reaction to some other stimulus than that induced by the graft.

The preceding discussion has been confined to conditions obtaining in the wood. The bark exhibits abnormalities fully as great. Both the intervention of cork layers between the phloem of the stock and scion and distortion comparable with that described have been noted. It is conceivable that interference with translocation may be one of the major factors in the failure of certain combinations to make satisfactory unions. Irrespective of the various theories of the path of translocation of foods and nutrients, there is adequate evidence that in certain unions the movement across the union would be expected to be difficult. That such an interference does exist is supported by the occasional appearance of swellings above the union, such as would be caused by ringing.

The observations recorded here are in no sense to be considered a complete inventory of the structures to be observed in grafted material, even of the combinations considered. From the limited number of grafts of a single combination sectioned, it is evident that there is a high degree of variability to be found between individual

specimens of a given combination. It would seem that an extensive study of the variations encountered in a single combination, with a

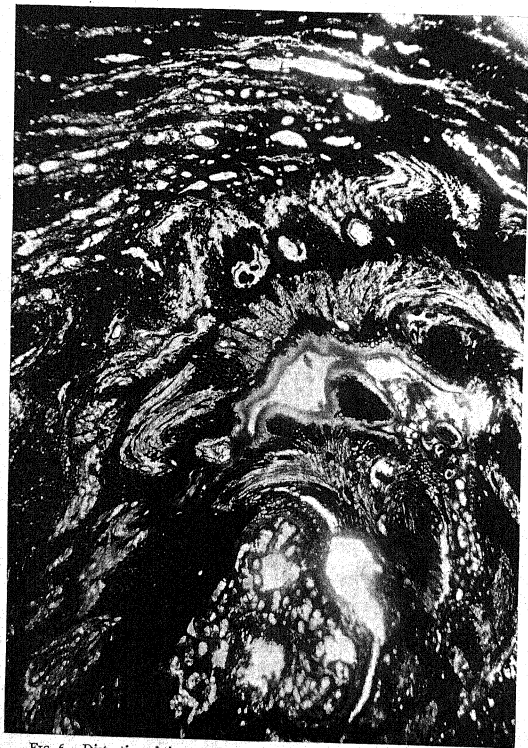


FIG. 6.—Distortion of tissue accompanied by gum formation at union of Japanese plum on almond; tangential section.

concurrent search for the factors inducing the variations, would prove profitable.

The combinations which have served as the basis of these notes are the following:

PRUNUS.—On Myrobalan (*P. cerasifera*):

Orange Cling and unknown peaches (*P. persica*)

Blenheim apricot (*P. armeniaca*)

Sugar and Imperial prunes (*P. domestica*)

Tragedy plum (*P. domestica*)

Beauty, Santa Rosa, and Formosa plums (*P. salicina*)

On apricot (*P. armeniaca*):

Santa Rosa plum (*P. salicina*)

Sugar prune (*P. domestica*)

Palora, Phillips, and Muir peaches (*P. persica*)

On Muir peach (*P. persica*):

Beauty plum (*P. salicina*)

I.X.L. almond (*P. communis*)

French prune (*P. domestica*)

On almond (*P. communis*):

Unknown almond

Beauty plum (*P. salicina*)

On Sweet cherry (*P. avium*):

Italian prune (*P. domestica*)

PYRUS.— On apple (*P. malus*):

Apple

Bartlett pear

Quince

On French pear (*P. communis*):

Bartlett pear

Unknown apple

On Japanese pear (*P. serotina*):

Bartlett pear

Quince

Apple

On *P. ussuriensis*:

Bosc pear

Bartlett pear

On quince (*Cydonia oblonga*):

Bartlett pear

The writer wishes to acknowledge his indebtedness to Mr. M. J. HEPPNER for his cooperation in supplying many of the unions sectioned, and to Dr. W. P. TUFTS for valuable suggestions in the preparation of these notes.

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## CHANGES IN SIZES OF YEAST CELLS DURING MULTIPLICATION

OSCAR W. RICHARDS

(WITH FIVE FIGURES)

During the period of rapid proliferation in the early growth of a population of yeast in test tube culture the cells are of nearly uniform size. As the toxic excretion products accumulate the larger buds are selectively injured, and the population is then composed of large resistant cells and small attached buds. In an effectively constant medium the distribution of cells of different sizes is unimodal. The changes in the sizes of cells are described in detail in this paper.

The diffused nucleus of yeast<sup>1</sup> prevents accurate measurement of the size ratio of the nucleus to the cytosome, so that it is not possible to determine the relation between this ratio and the initiation of budding. The writer's measurements show that the process of bud formation bears no relation to the size of the mother cell. The method proposed by BECKING<sup>2</sup> for the analysis of the size changes of cells during increase is not applicable to the measurements of yeast without great modification. SLATOR<sup>3</sup> followed the increase of yeast on a warm slide, and CLARK<sup>4</sup> photographed the cells, but neither measured the size of the individual cells.

### I

Two different strains of *Saccharomyces cerevisiae* were used in these experiments, and they will be referred to by the name of the

<sup>1</sup> The investigations on the histology of the yeast cell are summarized in GUILLIERMOND, A., *Les Levures*. Paris. 1912.

<sup>2</sup> BECKING, L. B., and BAKER, L. S., *Studies on growth*. Stanford Univ. Publ. Biol. Sci. 4:135. 1926.

<sup>3</sup> SLATOR, A., *Some observations on yeast growth*. Biochem. Jour. 12:248-259. 1918.

<sup>4</sup> CLARK, N. A., *Rate of formation and yield of yeast in wort*. Jour. Physical Chem. 26:42-60. 1922.

place where each was used, as Oregon data and Cambridge data.<sup>5</sup> The origin of the yeast and the technique used in measuring growth have been described.<sup>5</sup> The cultures were kept at  $30^{\circ} \pm 0.8^{\circ}$  C. in an incubator. This is the customary temperature for yeast culture; but evidence, to be published later, indicates that if this temperature of  $30^{\circ}$  is maintained within a few hundredths of a degree, it is critical for the budding of yeast. The latitude of variation of the usual laboratory incubators is so great that the cultures are not kept at the critical thermal point long enough for the critical effect to influence the growth to an appreciable extent.

The measurements were made with a planimeter from tracings of the outlines of the cells. Photomicrographs were enlarged with an Edinger drawing apparatus to give a total magnification of three to four thousand times before they were traced. The cells are almost spherical, so that a sharp image is formed only when the greatest diameter is in focus. Only those cells whose photographs were sharp were measured. The measurements are given in arbitrary area units, and the volumes of the cells are almost proportional to the three-halves power of the area measurements. The error of projection and measurement is about 5 per cent, as determined by making two independent measurements of the same group of cells.

The photomicrographs were made by placing a pocket kodak on top of the microscope. The mounting of the f. 6.9 lens just fits within the rolled edge of the ocular and makes a light-tight mounting. The difference in focus between the eye and the film was corrected for with a -9 diopter lens.<sup>6</sup> The yeasts were photographed in a haemacytometer, so that the ruling would indicate the magnification. The magnification of the image on the kodak film was about  $150\times$  when the 4 mm. objective and a  $10\times$  ocular were used. In the later experiments the advantage of the autographic feature was sacrificed

<sup>5</sup> RICHARDS, O. W., The growth of the yeast *S. cerevisiae*. The growth curve, its mathematical analysis and the effect of temperature on the yeast growth. Ann. Botany 42:11-14. 1928.

———, Potentially unlimited multiplication of yeast with constant environment and the limiting of growth by changing environment. Jour. Gen. Physiol. 11:525-538. 1928.

<sup>6</sup> Using this suggestion from FOOT and STROBEL, quoted in GUYER's Animal microscopy. Chicago. 1921 (p. 150).

for the greater ease of handling and the less expense of moving-picture film. A suitable insert reduced the opening in front of the film to twice the size of the standard movie exposure.

## II

The changes in the size of the cells during budding were followed by placing a few cells in nutrient fluid in the counting chamber and sealing the edges with vaseline. The cells were kept in an incubator at  $30^{\circ} \pm 0.8^{\circ} \text{C.}$ , except for the two minutes necessary to photograph them at the end of each 30-minute period. The cultures grew well and no unfavorable changes were observed before nearly 24 hours. Seven groups of cells were measured, each containing 2-11 cells.

The changes in size of a typical group of cells are shown in fig. 1. The growth, or increase in size, is made by the buds. The changes of the cells which are not budding are on the average less than the error of measurement and are not significant.

The average decrease in average cell size in the 30-minute interval preceding bud formation is 15.8 per cent. The cell giving off the bud decreased, on the average, 15.5 per cent in area units during the same interval. This shows that other cells which remain attached together in the group are not involved during the formation of the bud. During the same interval the growth increment in total area of all of the cells in the group was found to be about 8 per cent. The unaccounted-for size of the bud amounts to an average of 72 per cent, which must come largely from the imbibition of water.

BECKING has devised a method of studying cell size changes, assuming the cell growth to be a discontinuous process. These changes he describes objectively with tri-dimensional graphs which he calls clivoids. A clivoid showing the distribution of the sizes of the yeast group of fig. 1 is shown in fig. 2. This clivoid shows two growth periods without cell division, and is not comparable directly with any of the cases in BECKING's paper. His class VII, for cell division without cell growth, under unbalanced conditions, most nearly approaches the observations on yeast. His treatment could be extended to include the present observations for growth of the bud and not of the mother cell. The labor involved in the further mathematical development does not seem justified until more measurements of the changes in cell size are available.

## III

The average size of the cells present during the growth of a population of yeast follows a regular cycle. The average size decreases

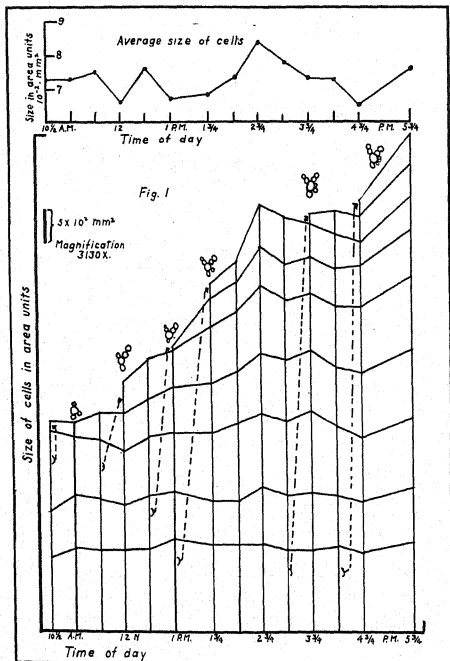


FIG. 1.—Changes in cell size during growth of a group of yeast

during the first 20 hours' growth after seeding, and then increases. The smallest size is found at the time that cell proliferation is greatest. The curves of average size are similar for the two strains meas-

ured, as shown in figs. 3*a* and 3*b*. Each point in fig. 3*a* is the average of ten measurements, that of fig. 3*b* is the average of thirty measurements, and each point in fig. 3*c* is the average of twenty-five measurements. This cycle of size change is not significantly altered if the acidity of the medium is maintained almost constant, but it is different if the environment is kept effectively constant in other respects (fig. 3*c*).<sup>7</sup> The asymmetry of the cycle is probably due to the changes in the environment produced by the accumulation of excretion products.

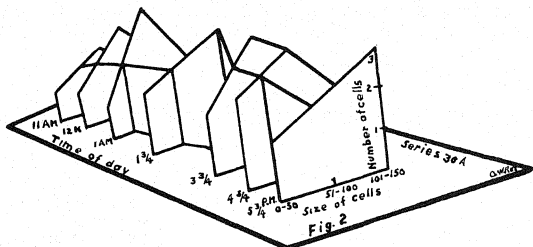


FIG. 2.—Clivoid showing distribution of sizes of cells shown in fig. 1, with respect to time.

The distribution of the sizes of cells present at different times is shown in figs. 4 and 5. In fig. 4 the average cell size (cf. fig. 3*a*) is indicated by the partition extending along the time axis. The distribution of the cells comprising the seeding depends on the age of the culture. During the rapid growth of the first 20–30 hours, more smaller cells are present and the distribution is practically unimodal. As the rate of growth of the population is gradually retarded by the accumulation of excretion products, the distribution becomes bimodal. This is most pronounced at about 100 hours after seeding, when the population is just maintaining itself at an equilibrium number.

The description of the yeast clivoids suggests a recurrence of cell

<sup>7</sup> Compare the growth curves given in<sup>6</sup> fig. 4*a*. The September observations of this figure are on the cells measured for figs. 3*c* and 5 of the present paper.

sizes such as would be expected with discontinuous growth, which BECKING emphasizes and which resembles his case III, when growth is a function of and dependent on cell size. The asymmetry of the

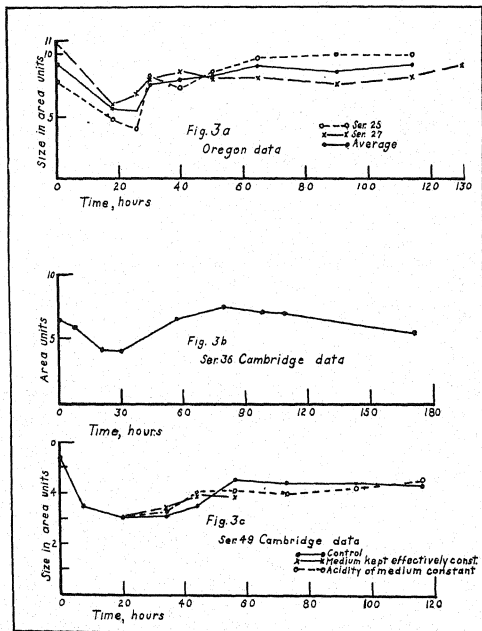


FIG. 3.—*a*, average size of cells (Oregon data); *b*, average size of cells (Cambridge data, series 36); *c*, average size of cells (Cambridge data, series 49).

recurrence suggests that it is not fair to apply the theory unless the effects due to changing environment are avoided. The tri-dimensional graphs indicate objectively the accumulation of these environmental effects.

The number of cells budding at the time of most rapid growth of the population is 22 per cent. At the period of equilibrium growth, about 100 hours, this is only reduced to 19 per cent. The difference is hardly significant, and shows that the mature cells are able to resist the toxic changes in the environment and to continue

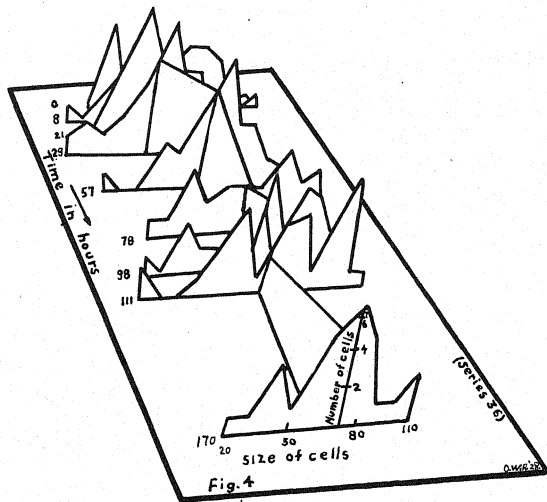


FIG. 4.—Clivoid showing distribution of sizes of cells (Oregon data, series 36)

to bud actively. The larger buds are selectively affected, and are apparently destroyed as soon as they are independent of the mother cell and before they are able to acquire resistance. This seems to be the explanation of the bimodal size distribution at this time. Much later, at 170 hours after seeding, budding practically ceases and the distribution of sizes again becomes unimodal.

The distribution of sizes of cells present in the population grown in a medium maintained at an almost constant acidity does not

depart significantly from that characteristic for the control population (fig. 5). The population grown in an effectively constant environment contains a unimodal distribution of cells until nearly the time that the experiment must for technical reasons be discontinued. The bimodality of the last time measured, 56 hours, is due apparently to a slight accumulation of waste products that has not reached a sufficient concentration to cause a decreased rate of multiplication. This probably accounts for the slight decrease in the average size of the cells (fig. 3c<sup>8</sup>). Up to this last observation there is no indication of a cyclic change in the average cell size.

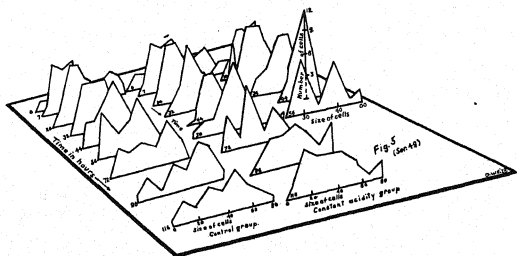


FIG. 5.—Clivoid showing distribution of sizes of cells (Cambridge data, series 49)

The distribution of the sizes of yeast growing in an effectively constant environment at a constant rate does not fit in any of the cases proposed by BECKING. These experiments suggest that the recurrence of the size modes of the cells is due to changes in the environment rather than to the fact that the cells regularly pass into the next larger size group, as would be the case if cell division were a direct result of cell size. Unfortunately, the technical difficulties prevented keeping the cultures in an effectively constant medium for a longer time. These observations give no indication that budding is a function of the size of the cells. Budding seems to be determined by the physiological condition of the larger cells, which are less affected by changes in the culture medium.



### Summary

1. The changes in the distribution of the sizes of cells with time are described for the growth of a population of yeast.

2. These changes follow a definite cycle, which is associated with the accumulation of toxic excretion products in the culture medium. When the medium is kept effectively constant the cycle does not occur.

3. The method proposed by BECKING, for the analysis of growth as determined by the size of the cell that is about to divide, is shown not to be applicable, without fundamental modification, to the analysis of the growth of yeast.

4. Bud formation is independent of the size of the mother cell, and seems to be determined by the physiological condition of the larger cells, which are less affected by changes in the culture medium.

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*[Accepted for publication February 12, 1928]*

## EFFECT OF SUNLIGHT ON SAP CONCENTRATION OF CITRUS LEAVES<sup>\*</sup>

F. F. HALMA AND A. R. C. HAAS

DIXON and ATKINS (2) and CHANDLER (1) have pointed out the effect of sunlight upon the sap concentration of leaves of different species of plants. The samples of citrus leaves employed in previous work of HAAS and HALMA (3) were collected from the shady side of the trees, in order to avoid effects of intense sunlight upon the sap concentration. The usual method of gathering leaf samples from trees has been to collect leaves from any or all sides of the trees. In so doing, leaves have been obtained some of which were exposed to direct sunlight, while others were in dense shade. That a large error would result from such a procedure will be shown in this paper. Moreover, evidence will be presented to show that Eureka lemon and Valencia orange leaves respond differently to the photosynthetic process, and that a relation exists between this phenomenon and the anatomy and chemical make-up of the types of leaves used.

The material consisted of normal mature leaves, one or more years of age, collected from ten-year-old trees growing on the grounds of the Citrus Experiment Station, Riverside, California. At each sampling, two sets of leaves from each tree were taken, one on the shady and the other on the sunny side. In choosing leaves on the sunny side, care was taken to select leaves which were fully exposed to direct sunlight. On the shady side only leaves from dense shade were selected. The leaves were picked before 9:30 A.M., and as quickly as possible, the greatest interval in time between the first and the last sample never exceeding twenty minutes. As an additional precaution, the order of taking the samples was varied at each sampling. The preparation of the material and the determination of the sap concentration were the same as in previous work by HAAS and HALMA (3).

<sup>\*</sup> Paper no. 180, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

From table I it is clear that when the material is secured from the shady side, the lemon leaf sap is considerably more dilute than the orange leaf sap. Similar but much smaller differences were found in sap of leaves obtained from the sunny side. Moreover, it was found that on prolonged exposure to sunshine the differences in sap concentration between lemon and orange leaves may disappear entirely. Since the leaves of the two species were practically identical in maturity, we may infer that either the lemon leaf is more efficient in its rate of building up of photosynthetic products than the orange, or the rate of translocation or respiration in the orange leaf is greater than in the lemon leaf. That the increase in freezing point lowerings, brought about by the action of direct sunlight, is due to carbohydrate metabolism is seen in the fact that the ash content of the expressed sap remains virtually the same, regardless of whether the leaves were in shade or in sunshine.

The question arises as to whether the exposure to direct sunlight increases the rate of transpiration and thereby lowers the moisture content of the leaves, and consequently increases the sap concentration. Again this can hardly account for the great differences found, since the ash content (table I) is not affected by exposure to direct sunlight.

We may now return to a previous statement, to the effect that either the lemon leaf is more efficient in the building up of metabolic products than the orange, or the former has a slower rate of translocation than the latter. We have evidence tending to give support to the former view.

In cross-sections of lemon and orange leaves (52 each), of the type used in sap concentration studies, the percentage of the width of the section occupied by the palisade tissue was measured and the following values were obtained:

	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
Eureka lemon leaves .....	$29.4 \pm 0.38$	$2.82 \pm 0.27$	$9.59 \pm 0.92$
Valencia orange leaves .....	$23.8 \pm 0.31$	$2.30 \pm 0.22$	$9.66 \pm 0.93$

It is evident that, at least in the type of leaves used in this work, the palisade cells of the lemon leaf occupy more space than is the case with the orange leaf. Since the palisade tissue is the chief photosynthetic unit of the leaf, this fact strengthens the view that the

TABLE I  
EFFECT OF SUNLIGHT UPON CONCENTRATION AND ASH OF LEAF SAP

DATE 1927	EUREKA LEMON				VALENCIA ORANGE				INCREASED FREEZING POINT LOWERINGS OF VALENCIA ORANGE OVER EUREKA LEMON (° C.)	
	Δ		Difference (° C.)	Ash of 20 cc. sap	Δ		Difference (° C.)	Ash of 20 cc. sap		
	Shade	Sun			Shade	Sun		Shade	Sun	
September 13.....	1.387	1.592	0.205	.....	1.631	1.681	0.050	.....	.....	0.089
16.....	1.359	1.590	0.231	.....	1.801	1.911	0.110	1.5288	1.5284	0.321
20.....	1.317	1.568	0.251	.....	1.727	1.783	0.056	.....	.....	0.442
23.....	1.327	1.422	0.095	0.7136	1.604	1.741	0.047	1.4005	1.4031	0.215
26*	1.160	1.313	0.353	0.6123	1.566	1.696	0.130	.....	.....	0.319
October 13.....	1.394	1.560	0.232	.....	1.498	1.614	0.116	.....	.....	0.406
November 3.....	1.187	1.387	0.200	.....	1.397	.....	.....	.....	.....	0.183
								.....	.....	0.104
								.....	.....	0.210

\* Mature leaves of current season from same trees as samples of September 20.

lemon leaf is more efficient photosynthetically than is the orange leaf, provided the rates of translocation and respiration are similar in both.

It has been shown by HAAS and HALMA (3) that the leaf sap of the Eureka lemon contains more magnesium than that of the Navel or Valencia orange. In view of the fact that magnesium is a constituent of chlorophyll, differences in magnesium content of the sap may possibly be another index of the photosynthetic activity of the leaf. Further indirect evidence is furnished by the fact that leafy lemon cuttings root much more rapidly than similar cuttings from orange (4). In addition, unpublished data of HALMA bring out the fact that leaf cuttings of the lemon produce nearly twice as much dry weight of roots per unit weight of leaf as orange leaf cuttings, when grown under the same conditions. Investigations now under way indicate a relation between the percentage of the leaf cross-section occupied by palisade tissue and rooting ability of leafy cuttings of the species; leaves having greater percentages of palisade tissue root more readily. It is realized that the respiratory, photosynthetic, and translocatory processes are interrelated, hence further discussion on leaf efficiency requires additional data.

In conclusion, it is well to emphasize the importance of collecting samples under uniform conditions of illumination. This precaution does not apply only to sap concentration studies, but equally well to determination of photosynthetic products. Large errors are bound to occur if the important effects of differences in exposure to sunlight, here pointed out are ignored.

### Summary

1. Increases in the sap concentration of leaves of Eureka lemon and Valencia orange occur when the leaves are exposed to direct sunlight.
2. The sap of Eureka lemon leaves on exposure to direct sunlight increases its concentration more rapidly than does that of Valencia orange leaves.
3. Increases in sap concentration due to the action of sunlight on the leaves are due entirely to photosynthetic products, the ash of the sap remaining the same under both conditions.

4. A greater percentage of the cross-sectional diameter of the Eureka lemon leaves is occupied by palisade tissue than is found to be the case with the Valencia orange leaves. This, together with the fact that more magnesium is found in the sap of Eureka lemon leaves than in that of Valencia orange leaves, and the more rapid response of leafy lemon cuttings to vegetative propagation, lends support to the view that greater increase in lemon leaf sap concentration in direct sunlight over that of orange leaf sap may be due to a more efficient photosynthetic system.

5. The great variation in sap concentration of citrus leaves brought about by direct sunlight indicates that large errors may result through indiscriminate sampling.

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## COLEOPTILE OF ZEA MAYS AND OTHER GRASSES

GEORGE S. AVERY, JR.

(WITH TEN FIGURES)

Whether the coleoptile in grasses is to be interpreted as a single leaf, or as the result of the union of two organs, is still a matter for discussion. SARGANT and ARBER<sup>1</sup> favor the theory that the coleoptile is the equivalent of a pair of fused stipules. WORSDELL<sup>2</sup> substantially agrees, concluding that it is a ligular-like structure formed by the union of stipules. They base their interpretations principally on the presence of the two vascular bundles characteristically present in the coleoptiles of most grasses. These two bundles establish a double symmetry in the coleoptile. Recently, however, PERCIVAL<sup>3</sup> has shown that in the coleoptiles of an Indo-Abyssinian emmer wheat (*Triticum dicoccum* Schübl.) from two to six bundles may be present. He has construed the presence of these several bundles as evidence in favor of the coleoptile being a single leaf, and not double in origin.

Further evidence in favor of the single leaf theory, as set forth by PERCIVAL in *Triticum dicoccum*, is supplied by the writer's observations on some 15,000 seedlings of *Zea mays* L. The greater number of plants examined represented different strains of the varieties Golden Glow, Ninety-Day Yellow Dent, and Golden Bantam. In addition Dwarf, Liguleless, and Chinese Suckering strains were observed. Certain strains of the Golden Glow variety showed more than two bundles in the coleoptile. Two to five bundles were observed to be present (figs. 1-4). These additional bundles destroy the "double" symmetry of the coleoptile.

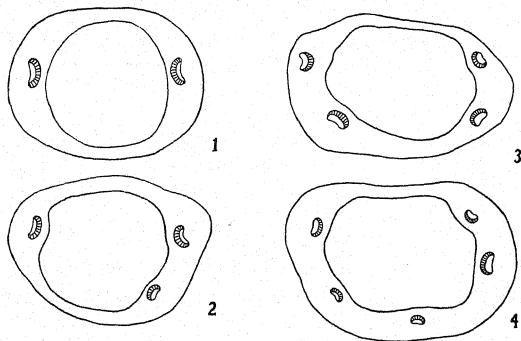
The position of the bud in the axil of the coleoptile should also be of some significance in determining whether it is a single structure,

<sup>1</sup> SARGANT, E., and ARBER, A., The comparative morphology of the embryo and seedling in the Gramineae. *Ann. Botany* 29:161-222. 1915.

<sup>2</sup> WORSDELL, W. E., Morphology of the monocotyledonous embryo and that of the grass in particular. *Ann. Botany* 30:509-524. 1916.

<sup>3</sup> PERCIVAL, J., The coleoptile bundles of Indo-Abyssinian emmer wheat (*Triticum dicoccum* Schübl.). *Ann. Botany* 41:101-105. 1927.

or represents the congenital union of two structures. If the bud is regularly alternate distichous with the buds that follow on the axis, it must bear the same relation to the coleoptile that later buds bear to the later leaves. If the coleoptile were actually a "double" structure, two buds might be expected in its axil instead of one, each bud in front of one of the two characteristic bundles. In no case was more than one bud found. In the cases of *Avena*, *Hordeum*, and *Triticum*, in all of which buds are usually found in the axil of the

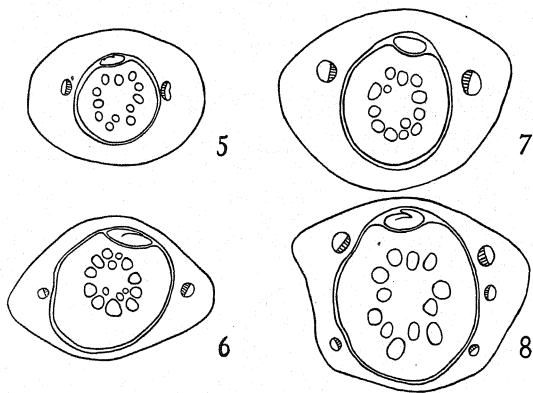


FIGS. 1-4

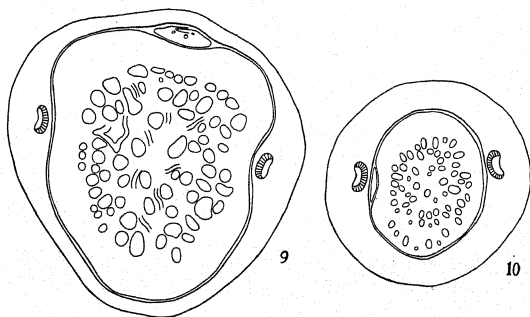
coleoptile, the bud occurs between the two bundles, on the same side of the axis as the scutellum and directly above its insertion (figs. 5-8). It has the same relative position with reference to the scutellum in *Triticum*, irrespective of the number of vascular bundles (figs. 7, 8). Buds are not commonly found in the axil of the coleoptile of maize. Of the large number of seedling plants examined, only three such buds were found, and these were in the Golden Glow variety. In no case was a bud found in the axil of a coleoptile that had more than the usual two bundles. In two of the three cases the buds occupied the same relative position as those in *Avena*, *Hordeum*, and *Triticum* (fig. 9). In the third case it occupied a position almost directly in front of one of the bundles of the coleoptile.



The fact that in one instance of maize a bud appeared in the axil of the coleoptile, almost directly in front of one of the bundles, does not seem significant. It will be noted that the buds observed in



FIGS. 5-8



FIGS. 9-10

the different grasses varied in position, being somewhat closer to one coleoptile bundle than to the other (figs. 5-8). A single case, such as shown in fig. 10, might be considered an extreme example of this variation. If the coleoptile is considered a single leaf, the bud in its axil bears exactly the same relation to it that later buds bear to later foliage leaves, except that the coleoptile has no midrib. The coleoptile is then alternate distichous with the later leaves.

There seems quite as much or more evidence to consider the coleoptile a single leaf, than the result of fusion of two stipules or other structures. If one considers the coleoptile the first green leaf of the plant,<sup>4</sup> it is not surprising that it differs from the later leaves. Such is also the case in a great many dicotyledons. In fact it is very often true that the first two or three leaves above the cotyledons differ markedly from the later leaves of the plant.

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<sup>4</sup> Under normal physiological conditions the cells of the coleoptile contain some chlorophyll, although such cells are usually confined to the vicinity of the vascular bundles.

## BRIEFER ARTICLES

### A STAIN COMBINATION FOR PHLOEM TISSUES OF WOODY PLANTS

(WITH ONE FIGURE)

Incident to research upon the phloem of our native woody plants, a Bismarck brown-Haidenhain's iron-haematoxylin combination has been found to give results more satisfactory than those obtained by the usual anilin safranin-haematoxylin schedule, and is therefore to be recommended. Fig. 1 is a transverse section of *Fraxinus pennsylvanica* Marsh, stained as indicated and photographed with a green ray filter at 250 diameters. The clearness of detail at this magnification, the stratification of the walls of the bast fibers, and the sharp demarcation between the sieve tubes and companion cells, ray cells, and phloem parenchyma cells should be noted. The dyes act as a triple stain, and have been used with equal success on the phloem of both hard and soft woods.

#### Staining schedule

1. Place sections in a 2 per cent aqueous solution of ferric ammonium sulphate for 20 minutes. If materials have been killed and fixed this time may be reduced by one-half.
2. Drain mordant and wash sections with five changes of distilled water to insure complete removal of excess mordant.
3. Flood sections with distilled water and add two or three drops of a 1 per cent aqueous solution of Haidenhain's iron-haematoxylin. Watch progress of stain under a microscope and stop at desired point by another change of water.
4. Drain off water and immerse section in a 1 per cent aqueous solution of Bismarck brown for three or four hours, depending on the thickness of the sections.
5. Drain off excess stain.
6. Dehydrate sections with the following changes of alcohol: 10, 20, 30, 50, 70, 95, and 100 per cent. Flood sections with two changes of 100 per cent alcohol to remove all traces of water.
7. Clear in xylol and mount in balsam.

The stone cells of hard woods stain a cherry red and the bast fibers a brilliant orange, the ray cells and other parenchymatous tissue a chestnut brown, and the middle lamella a dark blue. The bast fibers, parenchymatous tissues, and middle lamellas of conifers stain as indicated,

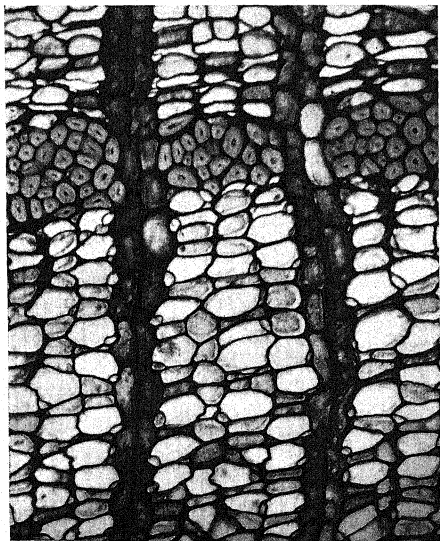


FIG. 1

while the stone cells turn a vivid burnt orange. Acknowledgments are due Dr. H. P. BROWN for suggestions in preparing the manuscript.—E. S. HARRAR, *New York State College of Forestry, Syracuse, N.Y.*

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## ORIGIN OF ENDODERMIS IN FERNS

In his recent contribution on the origin and development of tissues in the rhizome of *Pteris aquilina*, CHANG (3) draws certain fundamental conclusions on the cell lineage of the various morphological elements. He shows that the pericycle and endodermis are respectively the inner and outer products of the tangential division of a single layer of meristematic cells, and are accordingly sister tissues. This alone could hardly be considered sufficient evidence that the endodermis arises from the plerome. STRASBURGER (7), who also recognized the common origin of endodermis and pericycle, referred both of these layers to the periblem. CHANG, however, further determined that the common mother cell layer of endodermis and pericycle on the one hand, and the protophloem on the other, are derived from the periclinal division of a single layer of cells; hence he is certainly justified in his remark that "no room is left for doubt of the stelar origin of both the endodermis and the pericycle."

As CHANG recognized, however, this statement is in direct contradiction to the generalizations of the current textbooks. CAMPBELL (2), in discussing the rhizome of the Polypodiaceae as a group, repeats STRASBURGER's conclusion that both pericycle and endodermis are cortical in origin. JEFFREY (5), in describing the rhizome of *Pteris*, ascribes the endodermis to the cortex, and in such textbooks as those of STRASBURGER, and of COULTER, BARNES, and COWLES, the endodermis of the stem of vascular plants in general is treated under the heading of cortex, probably following the conclusions of VAN TIEGHEM and DULIOT. In consideration of these facts, it seems that CHANG would have been at pains to cite any work which lends support to his conclusion of the stelar origin of the endodermis. He does record the finding of SCHOUTE (6), confirmed by BARRATT (1), that in the stem of *Hippuris* the endodermis and several layers of the cortex are derived from the plerome; but, strangely enough, he neglects to mention the important monograph of CONARD (4) on the development of *Dicksonia* (*Dennstaedtia*) *punctilobula*. This writer demonstrated (in 1908) that the segments of the initial cell of the rhizome of this fern divide by periclinal walls into three layers, the outer one of which gives rise to the epidermis and cortex, the inner to the pith, and the middle to the plerome of the amphiphloic siphonostele. This latter divides by three periclinal walls to form four cells, of which the outer and the inner give rise respectively to the outer and inner endodermis and pericycle. The endodermis and pericycle, as revealed by actual mitoses as well as by the alignment of the mature cells, are sister

tissues which originate in the plerome. Thus we have independent evidence derived from two distinct genera of the Polypodiaceae with entirely different stelar types, the one siphonostelic and the other polystelic, of the stelar origin of the endodermis in the rhizome.

In the root of *Dicksonia*, on the other hand, the endodermis is cortical. The second pericline in each segment derived from the tetrahedral initial separates an inner cell which originates the plerome from an outer which gives rise to the endodermis and most of the cortex. The first periclinal division of this outer cell occurs near its inner wall, and cuts off the endodermis from the remainder of the cortex. The cortical affinity of the endodermis of the root has been demonstrated for a long list of ferns (including *Pteris aquilina*) which CONARD cites. In view of the direct continuity of the endodermis from the root where it is cortical, to the stem where it is stelar, it is evident that the sequence of cell walls in the apical meristem is of little importance in determining the subsequent morphological and physiological specialization of tissues.—ALEXANDER F. SKUTCH, *Johns Hopkins University, Baltimore, Md.*

[Accepted for publication January 27, 1928]

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# CURRENT LITERATURE

## BOOK REVIEWS

### Textbook of systematic botany

For many years the subject of systematic botany in American universities and colleges has suffered from the lack of a suitable text. This lack has been remedied in large part by a new volume by SWINGLE.<sup>1</sup>

The book is divided into two parts, the first part dealing with principles and methods, the second with a general discussion of the Spermatophyta and a special treatment of sixty representative families. The discussion of principles and methods is notable for its breadth of treatment and for its balanced perspective. It includes many phases of botany that at a former stage in the history of taxonomy were only too often neglected. Chapters upon evolution, principles of taxonomy, difficulties in classification, development of systems of classification, phylogeny of spermatophytes, and nomenclature serve to present the more theoretical aspects of taxonomy. These are followed by chapters of a somewhat more practical nature, dealing with the preparation of herbaria, also with the terminology and literature of systematic botany.

The volume is the outgrowth of some fifteen years' teaching by its author in a course given at the Montana State College. He appears throughout its pages to have kept in mind his intention to prepare primarily a textbook, and not a manual or some other more or less supplementary book. The general plan of presentation is excellent. The discussions are characterized by a commendable degree of fairness and conservatism. On the whole, it is probable that the introduction of this book to university and college classes will mark one of the most significant forward steps which the subject of taxonomy has taken in recent years.

As often happens with first editions, however, various inconsistencies or inaccuracies are present to detract from the value of the work. Some of these are apparently typographical slips. Thus VON BAER (p. 11) later becomes VON BAYER (p. 49). DECANDOLLE (p. 108) is DECANDOLE elsewhere (p. 29). This is hardly the case, however, with the many trivial names of geographic origin. Thus, *Asclepias Mexicana* (p. 200), *Rheum Rhaponticum* (pp. 146, 147), *Ulmus Americana* (p. 144), etc., have the trivial name's initial letter capitalized, while *Heliotropium peruvianum* (p. 203), *Magnolia virginiana* (p. 152), *Aeschynomene virginica* (p. 169), etc., do not. In *Xanthium* (p. 214) the writer appears to dis-

<sup>1</sup> SWINGLE, D. B., A textbook of systematic botany. pp. xiii+254. McGraw-Hill Book Co. 1928.

regard or overlook the staminate (and multiple) inflorescence entirely, and so attributes but two flowers to the inflorescence. VON BAER, already mentioned, is associated with a statement of the "law of recapitulation" (pp. 11, 49) in words that might more properly be ascribed to ERNST HAECKEL. The buds of *Salix* (p. 130) are confused with those of *Populus*. The nomenclature for specific names does not always conform to the latest findings of special research. Thus (p. 213) *Taraxacum officinale* is retained despite LAMARCK's earlier *T. vulgare*. BESSEY's view that opposite cauline leaves preceded alternate ones is correlated with the "fact that in dicotyledonous plants the first leaves (cotyledons) of the embryo are formed two at a node while in the monocotyledonous plants there is but one at a node." In view of researches tending to confirm the existence of a vestigial second cotyledon in various monocotyledonous seeds (mentioned by SWINGLE himself later on p. 220), the treatment given this matter appears inadequate and even misleading. One might wish also for more amplification in the chapter on nomenclature. Thus, for example, a discussion of homonyms and synonyms (p. 68) includes a treatment of *Lactuca integrifolia* Bigel., and *L. sagittifolia* Ell. is there given as the valid name to be adopted. SWINGLE might profitably add for the student's information that his reasoning in this case follows the American Code but directly violates the International Code, although indeed the pertinent article (no. 50) in the latter will doubtless be changed at some future date.

It is to be hoped that a second edition may enlarge upon certain subjects which are too much restricted in the first. Among herbaria, only one (Kew) outside the United States is listed (p. 75). In the chapter on literature there is no mention of some of the standard texts in systematic botany, such as WARMING's well known handbook. Under a heading, "The Classics" (p. 98), BENTHAM and HOOKER's *Genera Plantarum* is listed, but one looks in vain for several other fully as epochal works; such, for example, as DECANDOLLE's *Prodromus*. The subject of Descriptive Manuals (p. 102) is very inadequately handled, several works, such, for example, as TIDESTROM's *Flora of Utah and Nevada* having been omitted entirely. Under the heading (p. 113) of "Indexes, Catalogues, etc.," PRITZEL's *Thesaurus* is described, but his *Icones*, a much larger work, is omitted; nor are the monumental works of STEUDEL, PFEIFFER, and certain other writers listed. The gigantic card index issued periodically from the Gray Herbarium is omitted, as also several other invaluable card indexes and finding lists. Great library catalogues such as those of Kew and the Arnold Arboretum are likewise omitted, nor is there mention of such really extensive works as the BRADLEY *Bibliography* and the International Catalogue of Scientific Literature, to name only two of several. It would seem that an expansion of the volume in these respects could in no way impair its usefulness for the average class of beginners in the subject, but would serve to adapt it almost equally well to a large number of more advanced students who may have reached the point of entering upon research.—E. E. SHERFF.



## Microscopy of oil shales and coals

Two very useful books on the microscopy of oil shales and coals, and the technique of preparing thin sections and other microscopic preparations from these objects have recently been published. Their contents are of special interest to paleobotanists.

ROBERT POTONIÉ's volume on oil shales and related substances<sup>2</sup> summarizes our present knowledge of bituminous shales, of which the largest group is formed by the oil shales. He discusses the principal types of these shales, and accompanies his descriptions with ample microphotographic illustrations. The organisms which supplied the bituminous substances in the shales are discussed, and as far as possible determined. The author makes numerous original contributions and uses the literature very extensively. It is noted that many algal forms besides animal substances have produced oil in oil shales, and in some instances these algae can still be identified.

The microscopic examination of coal is discussed in detail by STACH.<sup>3</sup> He summarizes our present knowledge of the microscopic technique in coal examinations. The main chapter deals with the preparation of thin sections. The methods of LOMAX, THIESSEN, and JEFFREY are described in detail. There are also chapters on maceration and on microchemical investigation. The microscopic investigation with reflected light on the metallurgical microscope receives special consideration. There are chapters on microphotography of coal preparations, and on the microscopic determination of the various types of coal. The book is amply illustrated and contains a very complete bibliography.

Of special interest is a discussion of the three principal substances which constitute coal. These are: (1) the lustrous portion commonly called vitrit, which is primarily composed of wood; (2) the dull coal, durit, which contains primarily the exines and membranes of spores; and (3) the fiber coal, fusit, which originates from wood, like vitrit. Wherein the exact difference between vitrit and fusit lies has not yet been fully established.

The illustrations of STACH's book demonstrate how numerous, and in many instances how well preserved are the vegetable organs of which coal is composed. His treatise is an extremely interesting contribution to the study of botanical micropaleontology, a very important branch of a science which is now being greatly developed, namely, micropaleontology in general. Its zoological as well as its botanical side promises to be not only of great scientific value, but also to possess numerous practical applications in the study of our mineral fuels and of their origin.—A. C. NOÉ.

<sup>2</sup> POTONIÉ, R., *Allgemeine Petrographie der Ölschiefer und ihrer Verwandten*. pp. 173. figs. 27. Berlin: Gebrüder Borntraeger. 1928.

<sup>3</sup> STACH, E., *Kohlenpetrographisches Praktikum*. pp. 196. figs. 64. Berlin: Gebrüder Borntraeger. 1928.

### Filterable viruses

A very useful book for those interested in plant diseases, especially the so-called virus diseases, has been prepared under the editorship of RIVERS.<sup>4</sup> Although only one chapter applies immediately to plants, any one interested in the virus disease problem as found in plants will find it profitable to read the other chapters.

The book contains ten chapters. The first, by RIVERS, takes up the general aspects of filterable viruses. Filters and filtrations are discussed in the second chapter by MUDD. The third chapter, by CARREL, is devoted to a discussion of the use of tissue cultures in the study of viruses. COWDREY, in the fourth chapter, presents the intracellular pathology in virus diseases. Following these general topics, specific virus problems are discussed as follows: virus diseases of man as exemplified by foot and mouth disease and vesicular stomatitis, by OLITSKY; virus diseases as exemplified by contagious epithelioma (fowl-pox) of chickens and pigeons, by GOODPASTURE; virus diseases of insects (sacbrood of honey bees and the polyhedral disease), by GLASER; virus diseases of plants, by KUNKEL; and virus diseases of bacteria (bacteriophagy), by BRONFENBRENNER. Some of the chapters are well illustrated, and all carry extensive bibliographies. This feature alone makes the book a valuable contribution.

The volume is a most timely and welcome contribution in an interesting and perplexing field of biology, by men preeminently fitted for the task.—G. K. K. LINK.

### Physiology of bacteria

The first volume of a series by BUCHANAN and FULMER<sup>5</sup> on the physiology and biochemistry of bacteria has made its appearance. This volume will be of interest, not only to the bacteriologist, but also to the mycologist, for in spite of the title some attention is paid to those fungi which the bacteriologist classes as "molds and yeasts."

The book consists of five chapters, of which the first is a brief discussion of the scope of physiological bacteriology. The second chapter deals with the growth phases and growth rates of microorganisms in cultures. The remaining chapters are devoted to a discussion of the chemical composition, energetics, growth and movement of microorganisms, and the physico-chemical and physical characteristics of microorganisms and of their environment.

The authors have done a real service in very ably compiling and systematizing the extensive material relating to the physiology of microorganisms which has hitherto been widely scattered in numerous publications. The next volume is awaited with interest.—G. K. K. LINK.

<sup>4</sup> Edited by RIVERS, T., *Filterable viruses*. 8vo. ix+428. Baltimore: Williams and Wilkins Co. 1928.

<sup>5</sup> BUCHANAN, R. E., and FULMER, E. I., *Physiology and biochemistry of bacteria*. Vol. I. xi+516. *figs.* 78. Baltimore: Williams and Wilkins Co. 1928.

## NOTES FOR STUDENTS

**Mitogenetic rays.**—One of the most remarkable theories of recent years regarding cell division is that of the "mitogenetic rays" of GURWITSCH.<sup>6</sup> These rays are claimed to be emitted from actively dividing root tip cells, and to induce mitosis in neighboring roots. They are said to pass through air and quartz but to be stopped by glass and by a quartz plate which has been coated with gelatine. They are thus considered to be of the nature of ultraviolet light, and to have a wave length of 1900–2000 Angström units.

These conclusions are based upon the following type of experiment. The meristematic region of a horizontally growing onion root tip was placed a few millimeters from the same region of a root growing vertically downward. After at least twenty minutes the vertically placed tip was sectioned longitudinally, when many more mitoses were found on the side nearest the horizontal tip than on that farthest from it. These reports have not received much attention, probably because there seems to be no corroboration of them in our biological knowledge derived from other sources. It is highly important, however, that the truth about these rays should be established, and this appears to have been done in a recent paper. GUTTENBERG<sup>7</sup> first points out the inadequacy of GURWITSCH's own data for a critical examination of the problem. The zones studied were not exactly indicated, nor were the actual counts given in any complete or thorough manner. Figures were given in percentage of increase or numerical differences. Moreover details were not published as to actual phases of mitosis and the extent to which they varied in excess over corresponding stages on the opposite side of the root.

GUTTENBERG's own work appears to have been carefully conceived and elaborately carried out. *Pisum* as well as *Allium* was studied, isolated vertically growing roots were used as controls, and all mitotic phases were recorded separately. The first difficulty encountered was to determine accurately the beginning of prophase. Counts by different observers and by the same observer at different times convinced the author that this point could not be determined objectively; hence a great source of error arose in early prophase counts. Spireme, metaphase, anaphase, and telophase of course could be detected with certainty.

A point of note is made regarding median sections. Here many more cells (and so more nuclei) are seen than in tangential sections, and so more cases of division. Thus with higher totals greater deviations in number are to be expected. This would not give a greater percentage difference, however, and on this basis the results would be more accurate than when the totals are fewer.

<sup>6</sup> GURWITSCH, A., Das Problem der Zellteilung physiologisch betrachtet. Berlin. Verlag Julius Springer. 1926.

<sup>7</sup> GUTTENBERG, HERMANN VON, Die Theorie der mitogenetischen Strahlen. Biologisches Zentralblatt 48:31–39. 1928.

GUTTENBERG has made many thousands of counts, and has recorded the results in tables with phases shown separately. Both actual counts on the "induced" and opposite sides and differences between them are shown, with additional tables for median sections. From these it is apparent that as regards numbers of cells in any given phase, and totals of all cells definitely undergoing division, the differences between one side and the other are not significant. Sometimes there is an excess on one side, sometimes on the other. The only exception is in early prophase, where a greater number were more commonly counted on the "induced" side. This is the stage where the errors in determination are very great.

Further work was done on roots exposed from one and a half to two hours with fixation at various times thereafter, and also with more than one horizontal root. The results were similar to those given. Moreover, growing root tips were found not to affect photographic plates especially prepared for the range of ultraviolet light. GURWITSCH's claim that spectral rays of 1930-2370A are effective in inducing mitosis was not investigated, but the author feels that this also is not proved because of the method of counting. Moreover, if this should be established, it would not prove that roots emit such rays.

GUTTENBERG appears to have made his case. "Mitogenetic rays" are therefore due to join company with phlogiston, abiogenesis, "n rays" and other discarded theories. It is a great misfortune that mitosis, the process upon which the persistence of protoplasmic types depends, should remain so great a mystery in its fundamental principles. However, trial and error is the traditional method of advance, and it is devoutly to be wished that some element of success may soon crown our efforts.—R. O. EARL.

**Algae of Connecticut.**—HYLANDER<sup>8</sup> has published a very full account of the algae of Connecticut. The species are not described, but the numerous keys enable the reader to place the genera. Every county of the state was visited for material. It is recorded that "out of the 166 towns in the state, 123 have recorded algae stations." This certainly indicates a very thorough survey. The introductory pages give a full account of the morphology and classification of the algae. The classification is then presented under the following usual sections: Myxophyceae, Chlorophyceae, Phaeophyceae, Rhodophyceae. Under each species all the localities are cited where material has been collected. The display of algal flora in the state is very large, as shown by the great number of genera and species. This publication will serve for a long time as a standard of reference for students of this group in Connecticut.—J. M. C.

<sup>8</sup> HYLANDER, C. J., *The algae of Connecticut*. Conn. Geol. & Nat. Hist. Survey, Bull. 42. pp. 245. 1928.

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EFFECT OF PEAT MOSS AND SAND ON ROOTING  
RESPONSE OF CUTTINGS<sup>1</sup>

A. E. HITCHCOCK

(WITH PLATES V-VII AND FIVE FIGURES)

## Introduction

Studies relating to growth responses in plants have been conducted by numerous investigators over a period of many years. As a result of these investigations, many fundamental facts have been established regarding relationships between the plant and its environment. Similar relationships, however, have not been established for cuttings. For example, it is not known definitely to what extent external factors in a rooting medium may modify the tendency to initiation and growth of roots by cuttings. The fact that sand has been universally employed as a medium in which to root cuttings indicates that it has been generally thought that aeration and aseptic conditions are more important for the production of roots by cuttings than nutrient elements such as are present in soil.

MCCALLUM (14) attempted to prove that environmental influences cannot be regarded as directly causing the adventitious formation of roots or shoots. Light, gravity, and moisture were tested. Portions of the cutting were exposed to partially or completely saturated atmospheres as well as to water. *Phaseolus multiflorus* was mainly used for these experiments, although it was stated that *Salix*, *Heli-anthus*, *Taraxacum*, and *Tolmiea* responded in essentially the same manner. MCCALLUM concluded that regeneration of roots and shoots is determined by the same internal factors which control the devel-

<sup>1</sup> Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

opment of dormant buds. He regarded regeneration as inseparable from growth, and much of his work was concerned with the development of dormant shoot buds. His results do not adequately present or characterize the effect of external conditions on rooting response in a solid medium. So far as polarity is concerned, McCALLUM confirmed the work of VÖCHTING.

VÖCHTING (24) considered polarity of roots and shoots to be due mainly to properties inherent in living matter, although he states that light and moisture may have modifying effects. These conclusions reaffirm the results obtained in earlier experiments. His results show, in addition, that the roots from cuttings in sand were more numerous, larger in diameter, and longer than those from similar cuttings placed in water.

CURTIS (7) reported stimulation of root growth as a result of certain chemical treatments of cuttings prior to their placement in sand. The greatest stimulatory effect on root growth was produced by the use of a 1-2 per cent solution of potassium permanganate. Consistent results were obtained for this treatment in many tests on *Ligustrum ovalifolium*. Increased growth was not obtained, however, when the chemical was added to the rooting medium. Crone's and Knop's nutrient solutions in concentrations of 0.01, 0.10, and 0.50 per cent were found to be injurious to hard wood cuttings of *Ligustrum ovalifolium*.

SMALL (19) tested the effect of adding dilute acetic acid (1:10,000) to coconut fiber and to soil, and reported an increased percentage of rooting, or a reduction in time required for rooting, for all treated lots of cuttings as compared with the untreated ones. This treatment was found to be favorable for many varieties of cuttings. On the other hand, VIERHELLER (23) failed to improve the rooting of apple cuttings when SMALL'S acid treatment was used. PHILLIPS (17) likewise obtained no marked improvement in rooting when dilute solutions of acetic acid were added to sand, to soil, or to a synthetic leaf mold, in which cuttings of *Ocotea bullata* were placed. It would appear from these results that all kinds of cuttings will not respond in the same way to a given chemical modification of the rooting medium. PHILLIPS found, however, that when etiolated shoots of *Ocotea* were used for cuttings instead of normal shoots, a more favorable rooting response was obtained. Tests in sand, clay,

and synthetic leaf mold gave, in the order named, 30, 20, and 18 per cent rooting as compared with 6 per cent for the best lot of cuttings made from normal shoots. The physiological condition of the material selected for cuttings, therefore, is seen to be of considerable importance when comparing their ability to root in different media.

ZIMMERMAN (25) stated that peat moss was a good medium in which to root *Ilex*, Delaware grape, and some *Viburnum* cuttings. The last usually rooted at the base in sand, whereas in a peat moss medium rooting took place all along the portion of the stem which was buried. It was suggested that peat moss may contain stimulating substances which cause this specific response. The same worker also pointed out that favorable results might be expected with mixtures of peat moss and sand. These mixtures, it was stated, would hold water better than sand, and some benefit might be expected from the presence of peat moss.

SMITH (20) reported on the relation of acidity of the medium and root production in *Coleus*. Although *Coleus* cuttings rooted very readily in coconut fiber (pH 4.5-4.7), it was found that in a series of acid-alkali solutions ranging from pH 4.0 to 9.0, best rooting occurred at pH 7.0. In these solutions there appeared to be a direct relation between pH value and rooting response, if the dry weight of roots per gram of top dry matter was taken as the criterion of growth. SMITH inferred that the favorable rooting in acid coconut fiber was due to the efficient aeration provided by this medium. This conclusion was arrived at from the fact that the bases of cuttings in the fiber gave an immediate fat test with Sudan III, in contrast to a practically negative test for cuttings rooted in solutions. The presence of fatty substances at the base of the cutting was regarded as due to a plentiful supply of oxygen.

A preliminary report has been published on variation in rooting response of cuttings placed in media of different pH values (10). It was pointed out that *Azalea amoena* cuttings rooted well in natural peat moss, but rooted poorly in sand and in neutral peat moss. Cuttings of California privet gave the opposite response in these media. In a mixture composed of equal parts of peat moss and sand, both *Azalea* and privet rooted exceptionally well, indicating that such a mixture might prove favorable for many varieties of cuttings which root poorly in either peat moss or in sand.

KNIGHT and WITT (12) found that for rooting plum cuttings sand was more suitable than mixtures of coconut fiber and loam, loam and sand, or coconut fiber and sand. In these mixtures there was considerable variation in rooting for the four varieties tested, but in only one case was the percentage of rooting as high as that in sand.

The published data show clearly that root production by cuttings of one variety may be very different from that by cuttings of another variety, when both are placed in the same medium. Only a few varieties have been tested by any single worker, however, and comparable data by different workers are available for only a very small number of plant forms. It is evident, then, that before any generalizations can be made regarding the conditions which should obtain in a rooting medium in order to give favorable rooting, many different kinds of plants must be tested. Owing to the need for more specific information relating to this general subject, an experimental study of vegetative propagation was begun in 1925 at the Boyce Thompson Institute for Plant Research in Yonkers, New York. The purpose of this project is to determine, so far as possible, the important factors concerned in propagating plants vegetatively. Many phases of the project are now being studied. This paper reports the findings relating to the effect of peat moss and sand on rooting response of stem cuttings.

An attempt has been made by the writer to cover a wide range of plant types in making these media tests of cuttings. In the experiments reported 91 varieties of plants, including 46 genera, were used. In addition to obtaining data on differences in rooting response for many varieties of cuttings, an attempt has been made to determine to what extent the acid reaction of peat moss is concerned in producing favorable or unfavorable conditions for root production. The results here recorded are based on work which was done over a period of three years.

#### Materials and methods

Peat moss, sand, and mixtures of these two constituted the media used in the experiments.<sup>2</sup> Due to the marked contrast in their physi-

<sup>2</sup> Granulated peat moss was obtained from Atkins and Durbrow, New York City. Sand was obtained from the Yonkers Builders Supply Co., Yonkers, N.Y. The sand is reported to come from Cow Bay, Long Island, N.Y.



cal and chemical properties, as shown in table I, peat moss and sand afford unusually good means for obtaining widely divergent conditions without at the same time introducing more than two sets of substratum complexes.

Other phases of the work have shown that the capacity of cuttings to form roots is dependent to some extent upon the age of the wood, season taken, activity of growth, and the amount of leaf surface left on the cutting. These factors were accordingly taken into consideration when selecting material for the experiments. All leafy cuttings were brought into a turgid state, before placement in the rooting medium, by soaking in water for a few minutes or longer if necessary. In the greenhouse, pots were used in most cases to hold

TABLE I

PROPERTIES OF PEAT MOSS COMPARED WITH THOSE OF BUILDING SAND

PROPERTY	PEAT MOSS	SAND
Reaction (pH value) . . . . .	3.6	7.0+
Wt. of water in gm. held by 100 gm. dry weight peat moss . . . . .	1000*	20
Origin . . . . .	Sphagnum bogs	Rock strata
Organic matter content . . . . .	99 per cent	Negligible
Texture . . . . .	Finely fibrous	Coarse crystalline
Weight in gm. of 100 cc. (air-dry) . . . . .	15†	162

\* DACHINOWSKI gives a value of 1635.5.

† The moisture content of peat moss in the bale will vary greatly according to the humidity conditions under which it is stored.

the media; outside in the cold frames, cuttings were placed in beds of media protected by an overhead slat shade, no sash being used.

Since sand dries out readily when not covered by sash, an auto-irrigator was used in some of the experiments to provide the rooting medium with a higher and a more nearly constant moisture supply. The auto-irrigator consisted of a small clay pot placed inside of a larger pot, with sand or other media between the walls of the two pots. By keeping a supply of water in the smaller pot, the medium was assured of a continuous and a comparatively constant moisture supply. It was necessary for this purpose to select pots which lost water readily.

All hydrogen-ion measurements were made with a quinhydrone electrode apparatus of a type devised by W. J. YODEN, of the Boyce

Thompson Institute for Plant Research. Samples of the media were prepared by adding a sufficient amount of tap water to produce a few cubic centimeters of free liquid. These were allowed to stand overnight in stoppered flasks. A portion of the sample was then transferred to the electrode chamber by means of a small porcelain spoon. The sample thus measured, in the case of peat moss, was of the consistency of mush. Tap water was used, since preliminary tests showed there was no difference between the values obtained for samples made up with tap water and those made up with distilled water. On duplicate samples of standard buffer solutions the accuracy of the particular quinhydrone apparatus used by the writer was found to be within pH 0.03. When checked with a standard hydrogen apparatus, the agreement was within pH 0.03. A standard calomel half-cell was always used in making the measurements with the quinhydrone apparatus.

The reasons for using a heavy suspension of peat moss rather than an extract are explained in connection with the results showing the buffer capacity of peat moss. A heavy suspension appears to be more representative of the actual conditions in the rooting medium. Although OLSEN and LINDERSTRØM-LANG (16) found a filtrate from soil samples to be less variable than a suspension, they recommend using a minimum of water in making up samples for hydrogen-ion determinations. DOMONTOVITSCH (9) found that many plant juices give the same pH values regardless of whether or not the suspended particles were removed. Tomato leaves, however, gave higher pH values when the centrifuged extract was used than when the original extract was used. In comparing the hydrogen electrode with the quinhydrone electrode for measuring the pH values of plant juices, DOMONTOVITSCH found reasonably good agreement except for turnip and onion, in which cases there were appreciable differences in the readings.

BRAY (3) found that if the particles of a soil suspension were agitated by blowing in hydrogen gas from the bottom of the electrode chamber, the pH value was different from that obtained when the particles were allowed to settle out in the bottom of the vessel. These recent investigations have been particularly concerned with the part which solid materials play in affecting the measurement of

hydrogen-ions by the electrometric method. The tendency appears to be toward the use of suspensions rather than filtrates or extracts.

The time at which a reading should be taken after the quinyhydrone is added to the sample will no doubt vary according to the type of material being tested. This applies particularly to suspensions. CRUZ (6) took readings at the end of one minute. SNYDER (21) recommends from one-half to one minute for taking readings of the pH. Neither states why these time periods were chosen. As will be shown by the results obtained for buffer tests, an immediate equilibrium is not reached when quinyhydrone is added to a heavy suspension of peat moss.

### Experimental results

A general classification of rooting response for 96 varieties of cuttings is given in table II. Experiments recorded in tables III, IV, and V show the degree of rooting which was characteristic for each of the three groups listed in table III.

Experiment 1 (table III) shows that *Azalea amoena* cuttings rooted readily in natural peat moss and in a mixture of peat moss and sand, but that in neutral sand, neutral peat moss, and in a mixture of sand and neutral peat moss much poorer rooting was obtained. Eleven other experiments with *Azalea amoena* gave essentially the same results. Experiment 2 (table III) is mentioned primarily to show that neutral peat moss which has been previously used has different effects from those of a freshly prepared lot of the same material. Although *Azalea ledifolia* (*indica alba*) does not root so readily or so uniformly as *Azalea amoena*, the relative differences in the amount of roots formed in different media were found to be essentially the same. The difference in the size of the root systems for *Azalea amoena* cuttings rooted in peat moss and those rooted in sand is shown in figs. 1, 2. Uniformity of rooting in peat moss and lack of uniformity in sand are also evident in the same figures.

*Prunus glandulosa* rooted poorly in peat moss, exhibiting an injury similar to that of privet cuttings in the same medium. Excellent root systems were produced in sand, however, and in a mixture of sand and peat moss. These results are recorded in table IV (experiments 5 and 6). The results recorded for experiment 5 are also

TABLE II  
CLASSIFICATION OF VARIETIES OF CUTTINGS ACCORDING TO ROOTING RESPONSE IN PEAT MOSS AND SAND

GROUP I CUTTINGS ROOTED READILY IN PEAT MOSS BUT POORLY IN SAND	GROUP II CUTTINGS ROOTED READILY IN SAND BUT POORLY IN PEAT MOSS	GROUP III CUTTINGS ROOTED READILY IN EITHER PEAT MOSS OR IN SAND	
<i>Azalea amoena</i> * (6-12) † <i>Azalea ledifolia</i> * (8) <i>Azalea</i> (barky mollis hybrids) (7) <i>Bine spruce</i> (2-4) <i>Delaware grape</i> (7) <i>Enkianthus campanulatus</i> (9) <i>Ilex crenata</i> (3) <i>Vaccinium corymbosum</i> (6-8)	<i>Asclepias nivea</i> (1-4) <i>Berberis thunbergii</i> (8) <i>Carnation</i> (7) <i>Cotoneaster horizontalis</i> (7) <i>Daphne cneorum</i> (7) <i>Datura stramonium</i> (7) <i>Deutzia gracilis</i> * (6-8) <i>Heliotrope</i> (10) <i>Lagerstroemia indica</i> (8) <i>Ligustrum ibota</i> (var. <i>regelia-num</i> ) * (8) <i>Ligustrum japonicum</i> (9) <i>Ligustrum ovalifolium</i> * (6, 8, 11) <i>Ligustrum vulgare</i> * (8) <i>Mentha piperita</i> * (7-10) <i>Osmanthus aquifolium</i> (variegated) (8) <i>Prunus glandulosa</i> (pink variety) * (6-8) <i>Prunus tomentosa</i> * (6) <i>Prunus triloba</i> (12) <i>Rosa hugonis</i> (6) <i>Syringa vulgaris</i> * (6, 7)	<i>Buddleia davidii</i> (8) <i>Buxus sempervirens</i> (7, 11) <i>Callicarpa purpurea</i> (6-8) <i>Capsicum grossum</i> (4) <i>Coleus blumei</i> (4 var.) * (1-12) <i>Cornus florida</i> (pink) (6, 7) <i>Cornus florida</i> (white) (6, 7) <i>Cornus kousa</i> (6) <i>Cornus mas</i> (6) <i>Dahlia</i> (6 var.) (4, 5, 8-11) <i>Evonymus radicans</i> (7, 8) <i>Evonymus</i> (variegated) (7) <i>Forsythia intermedia</i> (5-9) <i>Forsythia viridissima</i> (5-9) <i>Fuchsia speciosa</i> (3 var.) (6-9) <i>Gardenia florida</i> (2, 7) <i>Geranium</i> (4, 6, 10-12) <i>Hydrangea opuloides</i> (8) <i>Hydrangea petiolaris</i> (6) <i>Ilex aquifolium</i> (8-10) <i>Ilex cornuta</i> (1) <i>Ilex glabra</i> (8-12) <i>Ilex opaca</i> (6, 8-12, 1) <i>Lonicera graefvittissima</i> (7, 8) <i>Lonicera morrowi</i> (7, 8) <i>Lonicera tatarica</i> (7, 8) <i>Philadelphus coronarius</i> (6-8) <i>Philadelphus folconeri</i> (6-8) <i>Philadelphus gordonianus</i> (6-8) <i>Philadelphus grandiflorus</i> (6-8) <i>Philadelphus leui</i> (6-8)	<i>Ribes alpinum</i> (6-8) <i>Ribes nigra</i> (6-8) <i>Rosa</i> (American Pillar) (6-9) <i>Rosa</i> (Dorothy Perkins) (6-9) <i>Rosa setigera</i> (5) <i>Rosa</i> (Silver Moon) (7) <i>Salix alba</i> (5, 6, 11) <i>Salvia splendens</i> (5, 8) <i>Sambucus canadensis</i> (6-8) <i>Spiraea</i> (Anthony Waterer) (6-8) <i>Spiraea arguta</i> (6-8) <i>Spiraea nipponica</i> rotundifolia (6-8) <i>Spiraea reevesiana</i> (6-8) <i>Spiraea thunbergii</i> (6-8) <i>Spiraea van houttei</i> (6-8) <i>Symphoricarpos racemosus</i> (6-8) <i>Symphoricarpos vulgaris</i> (6-9) <i>Taxus cuspidata</i> (1, 6, 11) <i>Taxus</i> (weeping variety) (1) <i>Tsuga canadensis</i> (6-8) <i>Ulmus parvifolia</i> (6) <i>Ulmus pumila</i> * (5, 6) <i>Viburnum carlesii</i> (7) <i>Viburnum opulus americanum</i> (6-8) <i>Viburnum opulus sterile</i> (6-8) <i>Viburnum tomentosum plicatum</i> (1-3, 12) <i>Weigela floribunda</i> (6-9) <i>Weigela rosea</i> (7)

\* Cuttings tested in neutral peat moss.

† Numerals in parenthesis indicate months during which cuttings were taken; for example, (6) refers to June.

Note: Cuttings in all three groups rooted readily in a mixture of equal parts of peat moss and sand, with the exception of the five varieties mentioned in the text.

TABLE III  
TYPICAL ROOTING RESPONSE FOR CUTTINGS OF VARIETIES LISTED IN GROUP I

No. of EXPERIMENT	VARIETY OF CUTTING	DATE	No. OF CUTTINGS IN EACH MEDIUM	RATING OF ROOT SYSTEM IN DIFFERENT MEDIA											
				Sand		Mixture sand and peat moss		Natural peat moss		Neutral peat moss		Mixture sand and neutral peat moss			
				Per- centage rooted	Size root system	Per- centage rooted	Size root system	Per- centage rooted	Size root system	Per- centage rooted	Size root system	Per- centage rooted	Size root system		
1.....	Azalea amoena	7-11 to 8-16-27	40	++ *	83	++ ++	90	++ ++	37	++	50	+			
2.....	Azalea amoena	8-28 to 10-18-27	30	++ ++	90	++ ++	97	++ ++	76†	++	90†	++			
3.....	Azalea ledifolia	8-28 to 10-18-27	30	++ ++	60	++ ++	56	++ ++	67†	++	60†	++			
4.....	Ilex crenata	2-28 to 3-28-27	20	++ 55	35	+	72	++ ++	.....	.....	.....	.....			

\* + represents a poor root system; ++ a fair root system; and +++ a normally vigorous root system.

† Medium which had been used previously and was 3 months old.

TABLE IV  
TYPICAL ROOTING RESPONSE FOR CUTTINGS OF VARIETIES LISTED IN GROUP II

No. of Experiment	Variety of Cutting	Date	No. of Cuttings in Each Medium	Rating of Root System in Different Media							
				Sand	Mixture sand and peat moss		Natural peat moss		Neutral peat moss		Mixture sand and neutral peat moss
					Per-centage rooted	Size root system	Per-centage rooted	Size root system	Per-centage rooted	Size root system	
5.....	<i>Prunus glandulosa</i> (pink)	6-25 to 8-10-27	15	++*	100	++	50	+	100	++	++
6.....	<i>Prunus glandulosa</i> (pink)	6-30 to 8-17-27	50	97	78	++	24	++	.....	.....	.....
7.....	<i>Deutzia gracilis</i>	6-15 to 8-6-27	20	100	100	++	50	+	.....	.....	.....
8.....	<i>Ligustrum ovalifolium</i>	7-1 to 8-11-27	20	70	100	++	30	+	.....	.....	.....

\* + represents a poor root system; ++ a fair root system; and +++ a normally vigorous root system.

TABLE V  
TYPICAL ROOTING RESPONSE FOR CUTTINGS OF VARIETIES LISTED IN GROUP III

No. of EXPERI- MENT	VARIETY OF CUTTING	DATE	No. of CUTTINGS IN EACH MEDIUM	RATING OF ROOT SYSTEM IN DIFFERENT MEDIA							
				Sand		Mixture sand and peat moss		Natural peat moss		Neutral peat moss	
				Per- centage rooted	Size root system	Per- centage rooted	Size root system	Per- centage rooted	Size root system	Per- centage rooted	Size root system
9.....	<i>Coleus blumei</i>	12-1 to 12-20-26	30	100	+	100	+	100	+	100	++
10.....	<i>Coleus blumei</i>	9-24 to 10-15-27	10	100	++	100	++	100	++	100	++
11.....	<i>Viburnum opulus sterile</i>	6-30 to 8-25-27	20	100	++	100	++	100	++	.....	.....
12.....	<i>Cornus florida</i>	6-16 to 7-26-27	15	35	+	83	++	35	+	.....	.....
13.....	<i>Ilex opaca</i>	9-15 to 11-16-26	15	54	++	85	++	67	++	.....	.....

\*+ represents a poor root system; ++ a fair root system; and +++ a normally vigorous root system.

shown in fig. 3. It is important to note that the best rooting of almond cuttings was obtained in neutral peat moss and in the mixture of sand and neutral peat moss.

Five varieties of cuttings listed under group II (table II) rooted poorest in peat moss, only slightly better in a mixture of sand and peat moss, and exceptionally well in sand. These varieties were *Deutzia gracilis*, *Prunus tomentosa*, *Daphne cneorum*, *Ligustrum japonicum*, and *Syringa vulgaris*. All other cuttings in group II rooted fully as well in a mixture of sand and peat moss as they did in sand.

Cuttings listed in group III rooted readily in both peat moss and in sand, although in most cases the best rooting occurred in a

TABLE VI

VARIATION IN PH VALUE OF CENTRIFUGED PEAT MOSS  
EXTRACT DUE TO DIFFERENT METHODS OF RINSING  
ELECTRODE AND ELECTRODE CHAMBER

RINSED WITH RUNNING TAP WATER (DUPLICATE LOTS)		RINSED WITH TEST SOLUTION	NO RINSING	RINSED WITH DISTILLED WATER
4.36*	4.22	3.99	3.94	3.94
4.51	4.49	3.94	3.94	3.94
4.65	4.49	3.94	3.94	3.94
4.41	4.65	3.94	3.94	3.94

mixture of sand and peat moss. Examples of this type are given in table VI. Ten other experiments with *Coleus* showed that in neutral peat moss by far the largest root systems were produced. For *Coleus* cuttings it was immaterial whether peat moss was neutralized by adding calcium carbonate, a calcium carbide waste product, an asbestos product, or by leaching with tap water.

A few experiments were conducted for the purpose of determining the effect of the medium on root growth after the roots had appeared. The results for mint cuttings are given in fig. 4. Similar results were obtained for *Coleus*, except that in this case the cuttings were not so sensitive to peat moss before being transferred. The check lots of cuttings were removed from the medium and replaced at the same time that duplicate lots were transferred from one kind of medium to another. These results show that the rate of root

growth in *Coleus* and mint cuttings is retarded by natural peat moss and accelerated by neutral peat moss.

Results of tests in which an auto-irrigator was used to supply moisture showed that root production could be improved by this

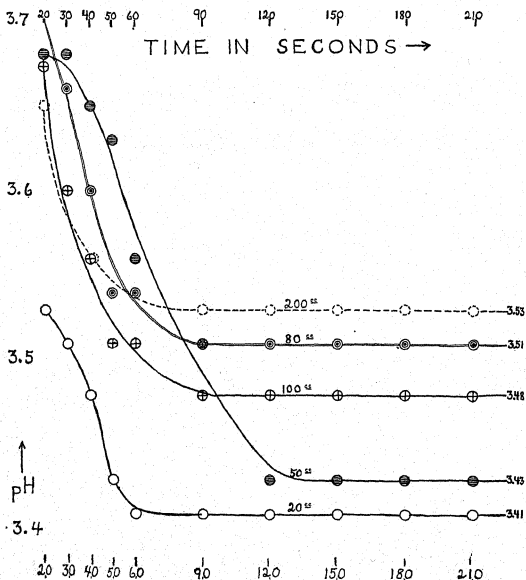


FIG. 7.—Equilibrium relations in electrode chamber for heavy suspension of peat moss, showing change in pH value with time at which readings were taken.

method, especially under conditions of high light intensity. The following cuttings were subjected to the auto-irrigator treatment: privet (3 varieties), *Prunus tomentosa*, Pillar rose, Dorothy Perkins rose, heliotrope, *Deutzia gracilis*, *Spiraea*, *Philadelphus*, *Asclepias*, and *Ilex opaca*. The results for two varieties of privet and for *Prunus tomentosa* are shown in figs. 5 and 6.



## BUFFER PROPERTIES OF PEAT MOSS

Difficulties first met with in measuring the hydrogen-ion concentration of peat moss made it necessary to learn more about the buffer capacity of this material. The data in fig. 7 show the variation in pH value that results when readings are made immediately after quinhydrone is added to a heavy suspension of peat moss in

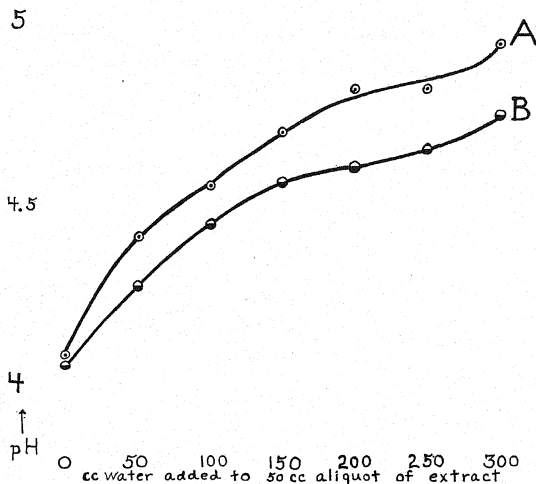


FIG. 8.—Change in pH of peat moss extract due to dilution

the electrode chamber. The change in all cases was in the same direction, that is, toward a more acid value. An equilibrium was practically reached at the end of one minute. Dilution values on each curve represent the amount of water added to 5 gm. of air-dry peat moss. For this particular lot the peat moss contained 50 per cent of moisture, although its moisture content in the bale varied from 30 to 150 per cent, according to the humidity conditions under which it was stored. The 20 cc. dilution curve represents the approximate

minimum moisture content at which a pH measurement of peat moss can be made with the quinhydrone apparatus.

Although the addition of water to peat moss, in great excess of that required to make up a sample for hydrogen-ion determination, does not alter appreciably the pH value of a heavy suspension, the dilution of a peat moss extract causes a marked change in pH value. The results for dilution of an extract are shown in fig. 8. Curves A and B represent dilution values for extracts from two different lots of peat moss. In both cases the extracts were freed from solid par-

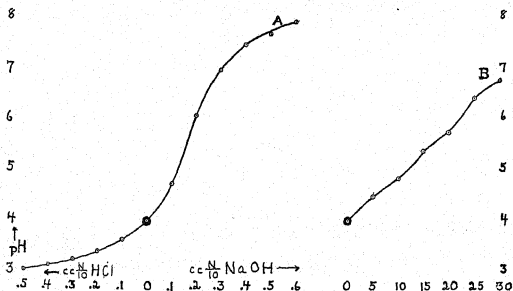


FIG. 9.—A, change in pH of peat moss extract due to addition to HCl and NaOH; B, change in pH of peat moss due to addition of NaOH.

ticles by centrifuging. These dilution curves show that a water extract is not very efficiently buffered. Further evidence to support this fact is given in fig. 9, which shows the result of adding N/10 sodium hydroxide and N/10 hydrochloric acid to 50 cc. portions of peat moss extract. Curve A (fig. 9) represents the change in pH value of a peat moss extract due to the addition of acid and alkali. Since only small amounts of either acid or base were required to cause an appreciable change in pH value, the extract cannot be considered as being efficiently buffered. The effectiveness of the solid material in taking out large quantities of base is shown by the results represented in B. In this case fifty times the concentration of base was

used, with the difference that the sodium hydroxide was added to the peat moss 24 hours before an extract was made.

In order to determine whether the capacity of peat moss to take up sodium hydroxide is a quantitative reaction, larger aliquots of peat moss and proportionally greater concentrations of base were used. Curves for these results are shown in fig. 10. *A* represents the change in pH value due to the addition of alkali in 5 cc. incre-

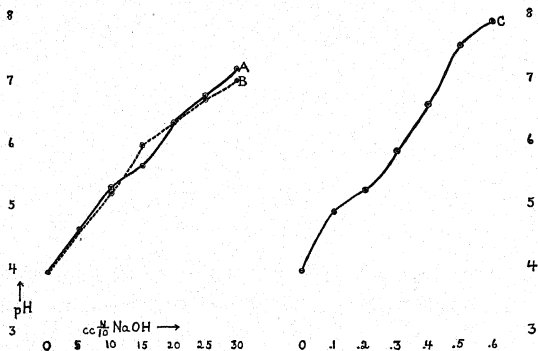


FIG. 10.—*A*, change in pH due to addition of NaOH to 50 cc. portions of peat moss; *B*, change in pH due to addition of four times the amount of NaOH to four times the volume of peat moss as used for obtaining values for *A*; *C*, change in pH of extract from 50 cc. of peat moss due to addition of NaOH.

ments to 50 cc. portions of peat moss. *B* represents the change in pH value due to the addition of alkali in 20 cc. increments to 200 cc. portions of peat moss. In both cases extracts were made 24 hours after alkali was added. *C* represents the change in pH value due to the addition of alkali in 0.10 cc. increments to the extract obtained from 50 cc. of peat moss. Although the same amounts of peat moss were used in obtaining extracts for values in *A* and *C*, the neutralizing capacity (*A*) is shown to be over fifty times that of its extract (*C*).

Since powdered calcium carbonate was usually employed to neutralize peat moss used in media tests, the effect of adding 1 gm. increments to liter portions is shown in fig. 11. *A* and *B* represent the change in pH value for an extract and a heavy suspension respectively, obtained from peat moss to which had been added 1

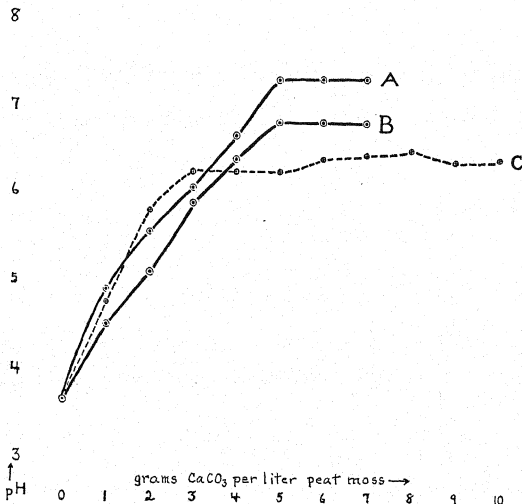


FIG. 11.—Change in pH of peat moss due to addition of increasing amounts of  $\text{CaCO}_3$ .

gm. increments of carbonate. Readings for these two curves were taken 5 days after addition of the carbonate. The results after 2 days for another lot of peat moss are shown in *C*. Higher pH values were invariably obtained for extracts than were obtained for heavy suspensions. This fact held true for both the natural peat moss and for that to which carbonate had been added. The time for complete neutralization with an excess of carbonate depends upon the method

of mixing in the carbonate. Ten grams of carbonate per liter of moistened peat moss will bring about neutralization in 24-48 hours if the material is thoroughly mixed and repeatedly squeezed with the hands. Sufficient water must be added to give the consistency of mush.

When the quinhydrone electrode and the electrode chambers were rinsed with running tap water, an error of considerable proportions was introduced. Rinsing with distilled water or with the test solution caused no error. Likewise, no error was introduced if the solution was completely thrown out of the electrode chamber without any subsequent rinsing procedure. Comparisons of different methods of rinsing are given in table VI.

#### Discussion of results

Contrary to the general belief that sand is the most suitable medium in which to root most kinds of cuttings, it was found that this held true under the conditions given for only 6 out of 96 varieties tested (table II). On the other hand, a mixture of peat moss and sand proved to be far superior to sand, inasmuch as 90 out of 96 varieties of cuttings rooted readily in this mixture. These results are of particular interest, since the advice in textbooks on propagation and in a recent paper by STEWART (22) is to the effect that "clean sharp sand" be used for rooting most kinds of cuttings.

The classification of cuttings according to their rooting response, as given in table I, applies in some cases only to cuttings taken within a limited period during the year, although most of the cuttings were taken from June 1 to October 1. *Prunus tomentosa* rooted readily when taken from May 15 to June 20, but much poorer rooting occurred after this period. *Cornus florida* cuttings taken in June rooted more readily than those taken at any other time. Maximum rooting for blue spruce occurred from February 15 to April 15. In contrast to the varieties just mentioned, *Azalea amoena* and California privet cuttings rooted at all times of the year. It is readily seen, then, that seasonal variation is of considerable importance in testing the effect of the medium on root production by cuttings.

In most cases species of the same genus responded similarly in a given medium. *Ilex crenata* and *Rosa hugonis* were exceptions to

this rule. Whereas the former rooted best in peat moss and the latter rooted best in sand, four other species of both *Ilex* and *Rosa* rooted best in a mixture of sand and peat moss. *Ilex opaca* taken in the fall appeared to root more readily in peat moss than when taken during December and January. *Daphne cneorum* was particularly sensitive to peat moss when young shoots were used, but with more mature shoots this effect was much less noticeable. Although blue spruce cuttings taken in late winter and early spring rooted best in peat moss, young shoots of the active spring growth were injured in the same medium. Such seasonal variations in rooting make a rigid classification of cuttings practically impossible, unless it be confined to a definite season of the year. Inasmuch as all of the varieties listed in table II were not tried at all times of the year, a complete seasonal classification could not be made.

For those cuttings which rooted best in peat moss, or in a mixture of peat moss and sand, the appearance of roots occurred at an earlier period, and the rate of growth was more rapid than for similar cuttings placed in sand. This relation held true for cuttings of woody plants but not for *Coleus* and mint, in which cases only a difference in rate of root growth was evident. Although in some cases, as for example with *Azalea amoena*, a fairly high percentage of rooting was obtained in the unfavorable medium, there were very marked differences in the size of the root systems, as illustrated in fig. 1.

Uniformity of root production in peat moss, in contrast to lack of uniformity in sand, is an important feature of the results shown in figs. 1, 2. Since conditions favoring rapid evaporation obtained in the greenhouse during the course of the experiment (August 28 to October 18, 1927), lack of uniformity in sand may be accounted for principally by variation in moisture conditions in different lots of the same medium. Variation in rate of water loss from different pots, with possibly unequal amounts of water added, was no doubt the cause of different moisture conditions in similar lots of sand. Peat moss, being such an efficient moisture retainer, does not dry out readily, however, and hence does not reach the critical low moisture content at which drying of cuttings takes place.

In connection with the subject of moisture content of the medium, it is of interest to note the results of tests by KNIGHT and WITT

(12). For fruit tree cuttings placed in soil under outside conditions, these investigators found that the best rooting was correlated with the least amount of rainfall, and that poorest rooting was correlated with the greatest amount of rainfall. These results are contrary to those obtained by the writer when using peat moss, sand, and a mixture of these two media. No doubt the general complexes of conditions are quite different in the two cases and different varieties of cuttings were used, so that a generalization based upon one set of conditions may not hold true for another. In contrast to the results obtained by KNIGHT and WITT for cuttings in soil, the writer found that a high moisture content, furnished by an auto-irrigator, favored root formation in many varieties of cuttings. Soft, succulent cuttings were usually rooted more readily than were more mature cuttings of the same plants. While this method was especially effective for sand, rooting in peat moss was also improved by auto-irrigation, more particularly when excessive evaporation conditions were provided such as are given by high light intensity, low humidity, and a relatively high temperature.

Cuttings which rooted poorly in peat moss usually showed definite signs of injury. Browning of the basal cut surface and sometimes the lower one-fourth of the cutting often occurred. This injury was particularly noticeable in the case of *Ligustrum*, *Syringa*, and *Prunus*. No callus was formed when this type of injury occurred. Partial or complete neutralization of peat moss allowed formation of good callus, and prevented the injury just described. It was necessary to decrease the acidity only slightly, that is, from pH 3.6 to 4.1, in order to prevent this type of injury. Hard wood cuttings of *Ligustrum ovalifolium* and *Syringa vulgaris* taken in the winter did not show this injury, and good callus formation was obtained on these cuttings even in peat moss of a high moisture content. It seems of interest to point out that in peat moss of a low moisture content (140 per cent) good callus formation occurs on many hard wood cuttings. At this low moisture content peat moss feels dry to the touch, yet a reading with a wet-and-dry bulb apparatus indicated a relative humidity of 96 per cent. The wet-and-dry bulb apparatus was buried in the peat moss, and the reading was made after a temperature equilibrium had been reached. Since

cuttings were rooted regularly in peat moss containing 500-800 per cent of moisture, it is readily seen that this medium furnished an unusually peculiar set of moisture conditions.

That the acidity of peat moss cannot be considered as the single controlling factor determining its value as a rooting medium is shown by fig. 3. Inasmuch as similar results were obtained with other cuttings listed in group II (table II), these results appear to be particularly significant. If the pH value of the medium were considered to be the limiting factor, there should be comparable root systems in lots A, D, and E, with lot B intermediate between lot C and lot A. Such responses did not occur. On the other hand, if mechanical texture such as provided by a mixture of sand and peat moss (lot B) were the limiting factor, then neutralization of the mixture should not have given the noticeable improvement shown in lot E.

It is difficult to explain why a difference of pH 0.6, the approximate difference in pH value between peat moss and a mixture of peat moss and sand, should allow such marked difference in rooting as was regularly shown. If it is assumed that the pH value of the mixture (4.1-4.4) is that at which maximum root formation occurs for cuttings listed in group II (table II), then a more favorable response should not occur in neutral media as is shown in lots D and E (figs. 3, 4). Such a complicated situation can be explained only on the basis that a combination of factors is operating to bring about a given type of rooting response. It is evident, however, that the pH value of peat moss is in some cases one of the most important factors, especially in connection with a direct injury to the cuttings, but in some cases affecting the rate of root growth. For cuttings which are not injured, however, the most important factor in peat moss appears to be the substances it contains (other than its acid reaction) which promote and maintain a rapid rate of root growth.

In addition to experiments with cuttings, the buffer tests on peat moss show that this material furnished conditions that are extremely difficult to analyze. Inasmuch as the hydrogen-ion concentration of peat moss varies according to the method of preparing samples for measurement, it is quite likely that the pH value as measured by the quinhydrone electrode, or by other means, does



not represent the actual hydrogen-ion concentration with which the cutting or roots have to contend. Regardless of the errors which may be involved when pH measurements are made on heavy suspensions of peat moss, it would appear that this type of sample is a more representative one than is an extract of the same material. The capacity of peat moss to take up such large quantities of sodium hydroxide, as shown by the titration curves, indicates that a solid medium such as peat moss must furnish a far more complex set of equilibrium relations than does a liquid medium which contains similar solutes.

Some varieties of cuttings rooted best when the reaction of the medium was comparable with that in which the parent plants are known to produce maximum growth. This is particularly true for *Azalea*, blueberry, lilac, and privet. While it is not the purpose of this report to follow out such comparisons, the results obtained indicate that cuttings of many plants will respond in a similar manner.

Peat moss and a mixture of peat moss and sand will leach sufficiently for a change of approximately pH 0.5 to occur during the course of an experiment, that is, in from six to ten weeks. Whereas the initial pH value of peat moss is 3.6, its final pH value at the end of an experiment would be 3.9-4.1. When the same peat moss was used again for privet cuttings, no marked injury resulted of the kind typical for freshly prepared peat moss. This fact further substantiates the idea that the critical pH value at which cuttings of privet are injured lies more specifically between 3.6 and 4.1. Although *Azalea* cuttings rooted readily in this type of "used" peat moss, root growth on privet cuttings was not so good as that obtained in a mixture, or in neutral peat moss. As was pointed out in experiments 2 and 3 (table III), *Azalea* cuttings rooted much better in a mixture of neutral peat moss and sand which had been previously used, than in a freshly prepared mixture. *Rhododendron* cuttings (*R. maximum*) formed excellent root systems in a leached peat moss which had previously been used in other experiments. The reaction of this medium four months after the original leaching, and at the time the *Rhododendron* cuttings were well rooted, was pH 6.76, showing practically no tendency toward reversion to the acid form. The results with *Rhododendron* are of particular interest,

since cuttings of this plant would be expected to respond the same as *Azalea*. COVILLE (4, 5) has shown that *Rhododendron* seedlings will grow well only in an acid medium, regardless of the amount or type of humus material present. It is possible, of course, that even though *Rhododendron* cuttings were readily rooted in a leached peat moss, they would not continue to grow for an unlimited period. Furthermore, even though root growth were favorable in leached peat moss, shoot growth might not be correspondingly good over a period of several years.

Neutralization of peat moss by alkaline reagents or by leaching with tap water causes the material to change from a light brown to a very dark brown, almost black. This change takes place within a day or two after the reagents have been added, and darkening continues as time goes on. If excessive amounts of alkali are added, the change in color is immediately produced. Change in color no doubt accompanies a chemical reaction. According to ITANO'S work (11), it seems quite possible that adjustment of the peat moss to a neutral reaction will allow of active bacterial decomposing action. The change just described may have some bearing on the difference in rooting obtained in used neutral peat moss.

*Ligustrum japonicum* rooted exceptionally well in sand. No roots were produced in peat moss during the time of the experiment. When the cuttings were transferred from peat moss to sand, however, roots appeared in two weeks' time. Such a result brings up the question as to whether the failure of root protrusion was due to lack of root initiation, or whether the root initials formed but failed to grow out through the bark. Although no anatomical studies were made on this material, it is believed that this phase of the general problem of vegetative propagation is extremely important. It is not definitely known whether for some kinds of cuttings different conditions in the rooting medium are required for root initiation from those required for root growth. Experiments with *Coleus* and mint cuttings indicated that for these particular plants differences in rooting in different media were due solely to factors which influenced the rate of root growth.

The efficiency of a mixture of peat moss and sand appears to be due mainly to its relatively high moisture-retaining capacity, to the

presence of growth-promoting materials furnished by the peat moss, to efficient aeration, and in some cases (*Azalea*, for example) to its acid reaction. The part which sand plays in this mixture (aside from that of dilution) is not clear. Both peat moss and sand are well aerated, so that efficient aeration in the mixture cannot be due to sand alone.

It appears from the results with cuttings that root growth may not be confined to such narrow ranges of pH values as has been supposed. Complex relations furnished by solid materials and many kinds of ions in rooting media must be considered as having a pronounced modifying influence on the extent to which the hydrogen-ion will exert its particular influence on root growth. SMITH's work on *Coleus* has an interesting bearing on this phase of the subject.

SMITH (20) found that root formation in *Coleus* cuttings attains a maximum in liquid media at pH 7.0, whereas in coconut fiber excellent rooting was obtained at a much more acid value (pH 4.5-4.7). This fact appears to be of particular importance, since the pH value of coconut fiber is comparable with that of a mixture of sand and peat moss. The writer found that in acid peat moss (pH 3.6) and in neutral sand *Coleus* cuttings rooted equally well. In the mixture of sand and peat moss (pH 4.1-4.4) root growth was invariably better than in either peat moss or in sand. The pH value of this mixture, as well as that of coconut fiber, was found by SMITH to be the acid limit (that is, pH 4.5) for *Coleus* cuttings placed in liquid media.

In view of these results with *Coleus*, it does not appear that data obtained for cuttings rooted in liquid media can always be used to explain the limiting factors for root production in solid media. SMITH's statement that, other conditions being equal, a neutral reaction of the medium was best suited for root production in *Coleus* cuttings, cannot be regarded as a very safe guide, for it is these "other conditions" which are often of the greatest importance. Differences in time of root protrusion at different pH values found by SMITH were not noted by the writer for *Coleus* cuttings placed in peat moss and sand. Observations by the writer included an examination of cuttings in pots of media and in glass tubes. In the latter case, cuttings of *Coleus* were placed next to the glass and fully

exposed to view. Roots appeared from cuttings in peat moss, in neutral peat moss, and in sand at the same time, at least during the same day, yet very marked differences in rate of root growth were observed thereafter in the different media.

Whether peat moss was furnished in its natural acid state, neutralized, or mixed with sand, a more rapid rate of root growth occurred in a medium containing peat moss than in one containing only sand. This brings up the question of the nutrient conditions which are furnished by peat moss. REID (18) pointed out that for tomato cuttings a low nitrogen value in the medium was more favorable for root growth than a high one. Such a result, however, was dependent upon the availability of carbohydrates in the cutting. Whether this relation holds true for other kinds of cuttings has not as yet been demonstrated. In order to gain some idea of the nutrient value of peat moss, tomato and buckwheat seedlings were grown in soil, natural peat moss, neutral peat moss, acid sand, and in neutral sand. Both of these species were able to grow to maturity in peat moss. The growth of tomato in natural peat moss was noticeably retarded during the first few weeks, but later the plant continued in what appeared to be a normal rate of growth, and set fruit. The tomato placed in neutral peat moss was at all times in a more vigorous state of growth than any of the others, including that in soil. Buckwheat did not show such marked variation in the different media, all plants setting fruit and attaining about the same vegetative growth. While this particular set of experiments cannot furnish any quantitative information, it shows that the nutrient value of peat moss is sufficiently high to be an important factor in determining the type of response in this medium.

BOTTOMLEY's experiments (1, 2) with "bacterized peat" showed that the products of bacterial action of peat would stimulate growth of certain plants, especially that of *Lemna minor*. Raw, unbacterized peat would not do this. In connection with this work it is interesting to note the results which ITANO obtained with a Michigan acid peat. The normal alkaline permanganate soluble nitrogen was increased from 5.9 to 71.5 after adjusting the medium to pH 7.0 and adding accessory food materials containing vitamin B. Whereas BOTTOMLEY obtained his "growth-promoting substances" (auximones) from bacterial action on peat, ITANO added "growth-pro-

moting substances" to peat in order to promote bacterial decomposition.

The variation in chemical and physical properties of different types of peat, as shown by DACHNOWSKI (8), makes a comparison of growth responses in various types of peat or peat moss media a somewhat doubtful procedure. No doubt the failure of some workers to repeat BOTTOMLEY's experiments has been due to the fact that the type of peat was different from that used by BOTTOMLEY. DACHNOWSKI makes a definite distinction between "peat" and "peat moss." The type used by the writer is designated "granulated peat moss." This is a standard commercial product which comes from bogs in Germany. It corresponds to the type which DACHNOWSKI classifies as a poorly disintegrated bog moss type (Section C, No. 7, p. 19). Over a period of three years this peat moss has proved to be of uniform texture and of the same acid reaction.

While the use of peat moss as a rooting medium for cuttings is by no means new, its use previous to 1925 for such a purpose in this country was noticeably limited. During the last two years, however, peat moss has been used rather extensively, not only as a medium in which to root cuttings, but also as a means of improving soil conditions. According to frequent reports in *Gartenwelt*, peat moss (torfmull) has been in more general use in Germany than in this country, but no attempt has been made to furnish detailed experimental data on comparative rooting responses of cuttings in peat moss and in sand.

### Summary

1. According to their rooting response in peat moss and in sand, 96 varieties of cuttings (including 46 genera) have been classified into three groups. Cuttings which rooted readily in peat moss but poorly in sand are placed in group I; those which rooted readily in sand but poorly in peat moss are placed in group II; cuttings which rooted readily in either peat moss or in sand are placed in group III.

2. The fact that cuttings in all three groups rooted readily in a mixture composed of equal proportions of peat moss and sand (with the exception of five varieties in group II) indicates that this mixture is superior to sand as a general medium in which to root cuttings. Although the pH value of the medium was an important factor in determining the type of rooting response of some varieties

of cuttings, it was not the single limiting factor. The critical acid value, at which injury to the cuttings listed in group II occurred, was found to lie between pH 3.6 and 4.1. For the same varieties of cuttings callus formation was inhibited at pH values more acid than pH 4.1.

3. Whether peat moss was furnished in its natural acid state, neutralized, or mixed with sand, a more rapid rate of root growth occurred in a medium containing peat moss than in one containing only sand. Good rooting occurred for most varieties of cuttings over an acid range of pH 4.5-7.0.

4. Uniformity of rooting response of *Azalea amoena* cuttings in peat moss is attributed to the efficient moisture-retaining capacity of this medium. An increased moisture content of sand, as furnished by auto-irrigation, showed that in many cases, but especially under conditions of high light intensity, a more favorable rooting response was obtained.

5. For *Coleus* cuttings the conditions in the medium influenced the rate of root growth rather than the time of root protrusion. Cuttings of *Ligustrum japonicum* which failed to root during two months in peat moss, rooted in two weeks when transferred to sand, indicating that root initiation had probably taken place, but that unfavorable conditions provided by peat moss prevented root protrusion.

6. The efficient buffer capacity of peat moss was found to be due principally to the solid material, and not to the solutes in an extract. Methods for preparing samples and for making pH determinations are described in detail. Extracts of peat moss were found to give higher pH values than heavy suspensions.

Experiments here reported were carried out in the laboratories of the Boyce Thompson Institute for Plant Research. The writer is greatly indebted to members of the staff of the Institute and to Professors R. A. HARPER and S. F. TRELEASE of Columbia University for helpful suggestions and criticisms given during the progress of the work.

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### EXPLANATION OF PLATES V-VII

#### PLATE V

FIG. 1.—*Azalea amoena* cuttings rooted in different media: A, peat moss medium (pH 3.6); B, bank sand medium (pH 5.8-6.2); C, Bay sand (Long Island, N. Y.) medium (pH 7.0+).

FIG. 2.—*Ligustrum vulgare* cuttings rooted in different media: A, peat moss medium (pH 3.6); B, bank sand medium (pH 5.8-6.2); C, Bay sand (Long Island, N. Y.) medium (pH 7.0+).

#### PLATE VI

FIG. 3.—Cuttings of *Prunus glandulosa* rooted in different media: A, sand medium (pH 7.0+); B, mixture of peat moss and sand (pH 4.1-4.4); C, peat moss medium (pH 3.6); D, neutral peat moss medium (pH 7.0-7.3); E, mixture neutral peat moss and sand (pH 7.0-7.2).

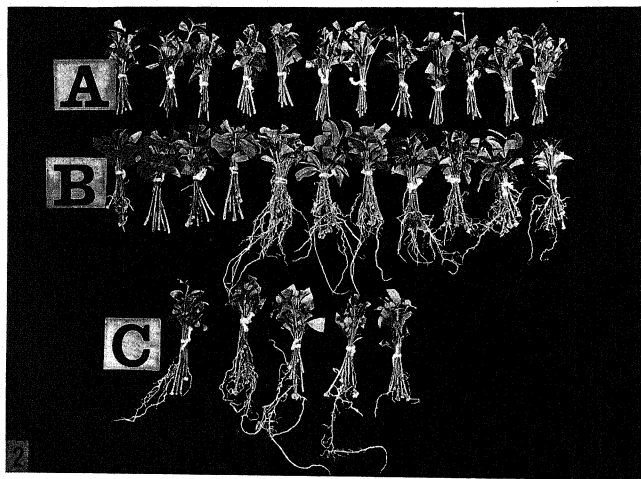
FIG. 4.—Cuttings of *Mentha piperita* rooted in different media: A, sand medium (pH 7.0+); B, peat moss medium (pH 3.6); C, neutral peat moss medium (pH 7.0-7.3); D, neutral peat moss (pH 7.0-7.3) for ten days, then transferred to peat moss (pH 3.6); E, peat moss (pH 3.6) for ten days, then transferred to neutral peat moss (pH 7.0-7.3).

#### PLATE VII

FIG. 5.—Cuttings of *Ligustrum vulgare* (top row) and *Ligustrum ibota* var. *regelianum* (bottom row) rooted in different media; effect of auto-irrigation also shown: A, peat moss medium not irrigated; B, peat moss medium auto-irrigated; C, neutral peat moss medium not irrigated; D, neutral peat moss medium auto-irrigated; E, sand medium not irrigated; F, sand medium auto-irrigated; G, sand medium not irrigated (this particular lot of cuttings was shaded daily until 1:00 P.M.).

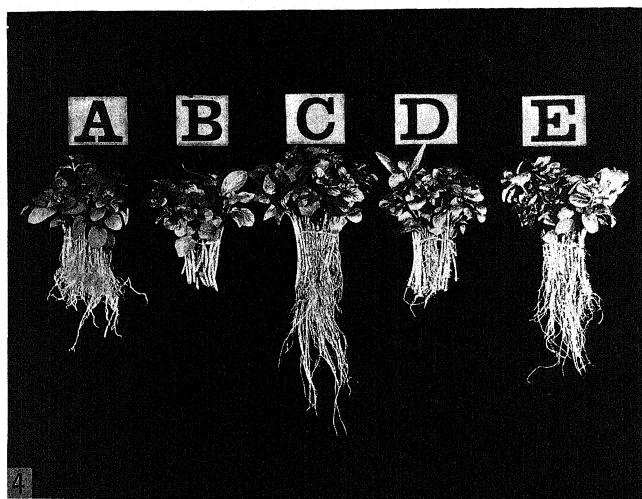
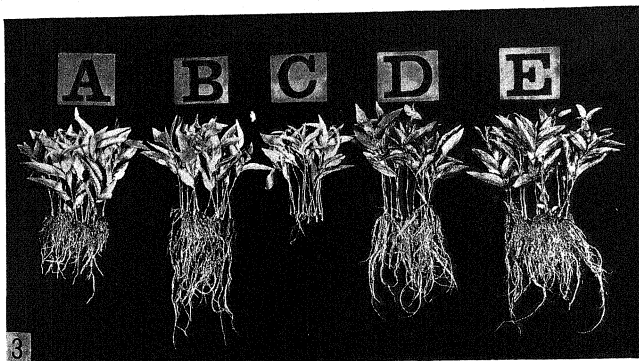
FIG. 6.—Cuttings of *Prunus tomentosa* rooted in sand: A, auto-irrigated; B, check (not irrigated).





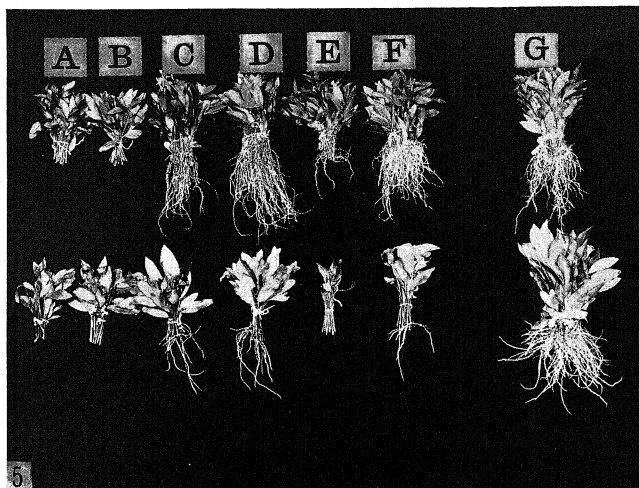
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# MORPHOLOGY OF SASSAFRAS IN RELATION TO PHYLOGENY OF ANGIOSPERMS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 383

GEORGIA V. COY

(WITH PLATES VIII, IX)

## Introduction

The question of the origin of vascular plants has stimulated both investigation and speculation, particularly in regard to the obscure beginnings of the Angiosperms. Botanists continue to investigate and speculate and they continue to be baffled, but slowly their lines of reasoning converge.

Paleobotany, once merely a study of outward form, in recent years has included more and more the investigation of internal structure. It has become most productive, and promises to be increasingly helpful in clearing up relationships. There is evidence of this, for example, in the publications on the Rhynie fossils of the Scottish Old Red Sandstones of the Devonian. These fossils have caused some shifting of the lower stretches of the phylogenetic lines, and seem to indicate at least the direction in which may lie their ultimate connections. ARBER (1) brought together the available material on Devonian fossil plants and divided them into two floras. The earlier and simpler of these, the Psilophyton type, were land plants with rootless rhizomes having dichotomously branching, but leafless, thalloid-like stems with terminal sporangia of several wall layers. These he considered progenitors of the plants of the Upper Devonian levels that made up his "Archaeopteris flora," and which were true though simple vascular plants, the most primitive Pteridophytes known. As a result of his studies, he derives all higher plants from the Algae by three independent lines, the Sphenopsida, the Lycopsida, and the Pteridopsida; with the Psilotales forming a fourth but much later one.

KIDSTON and LANG (21) have also published, among others, the description of a Devonian plant (*Hornea*) that indicates Bryophyte

affinities by its sphagnum-like sporangium with a columella and cuticularized spores in tetrads. It has a tuberous rhizome comparable with the embryonic protocorm of some of the Lycopodiales. Although the gametophytes are unknown, making comparison with Bryophytes correspondingly difficult, the investigators consider that the Rhynie fossils, so obviously related to the three great lower plant assemblages, have materially lessened the gaps between them.

SCOTT (25) calls attention to evidence from the Rhyniaceae indicating that the sporangium may be but a modified branch. KIDSTON and LANG (22) in fact refer to sporangia as representing remnants of Rhynaceous branch systems. This may cause some revision of ideas on sporophyll and sporangiophore, because the Devonian plants with their distinct terminal sporangia are regarded as leafless, not because of reduction, but because of their extremely primitive character.

### Ferns and seed plant line

The position of the ferns in the evolutionary sequence is somewhat variously regarded. With respect to the group itself there is a tendency, even in the face of the modern trend toward the polyphyletic idea (as for instance, in ARBER'S scheme) to consider them as representing development from a single line. In SCOTT'S opinion, the characteristics long regarded as favorable to this view, such as the prevalence of an alternation of generations that stresses the sporophyte phase, the uniform mode of development of reproductive organs, and a similarity of vascular and stomatal histology, have been augmented by the discovery of their possible ancestors among the Devonian plants. He thinks, moreover, that the ferns are not in the direct line of Spermatophyte descent, because, rather paradoxically, the Cycadofilicales are too nearly like the Filicales to have been their descendants. Their great similarity would necessitate, in that case, an affinity too close to permit of the important differences between them, such as heterospory and the seed habit. The evidence of history seems to support this view, in that there are Middle Devonian Cycadofilicales or Gymnosperm stems (*Paleo-pitys*); but there are no records of ferns in the Early Devonian,



where they would naturally be expected if they had given rise to the higher forms.

Among those who regard the ferns as the progenitors of the seed plants is BERRY (9), who considers that Pteridophytes must have developed heterospory early in their history, because of the fact that the Cycadofilicales were undoubtedly derived from ferns of at least Pre-Devonian age. COULTER and CHAMBERLAIN (12), while granting that a possible Lycopod descent is worthy of consideration, hold the Gymnosperm derivation through *Gnetum* as untenable, and through Coniferales as unlikely, but look upon the early heterosporous Pteridophytes, with their generalized conditions of stem anatomy and reproduction, as a favorable point of departure for the Angiosperm line.

On the other hand JEFFREY (19) believes that Monocotyledons and Dicotyledons have not come directly from any group of vascular Cryptogams, either living or extinct, since the only organ in which they show the characteristic features of the organs of Cryptogams is the root, a fact without significance since all roots are alike in that respect.

However opinions may differ as to the true place of the ferns, or in regard to the relationship of the Cycadofilicales to other plants lower in the scale, these primitive seed plants, admittedly a large and heterogeneous group, are thought to stand at the point of divergence of all the higher Spermatophyte lines. Emerging from this Cycadofilicales plexus, the two main evolutionary series, those of the Cycadophyte and the Coniferophyte succession, carry on seed plant development with affinities varying according to the interpreters.

### Seed plants

The Gymnosperms as a group have a long history and rather clearly indicated relationships, but this does not apply to the Gnetales, for which no fossil records have been found; a fact that, coupled with some resemblances to the Angiosperms, has made them the subject of much discussion. The characters which they display in common with the higher forms include the possession of true vessels in the secondary wood, companion cells in the phloem, a similar mode of sporogenesis and of fertilization, the reduction of

the gametophyte, some features of the embryo sac, embryo development, and, according to some, the floral morphology.

On the other hand, the researches of LANG and THOMPSON have shown them to be Gymnosperms. THOMPSON (29) finds that *Ephedra* shows conifer traits in the arrangement of the primary bundles, the double leaf trace, the disposition and structure of the pits on the tracheids, the bars of Sanio, tertiary spirals, lignified rays, wood parenchyma, and the endarch vascular bundles of the leaf. Their origin he considered to be near the base of the conifer line.

In view of the lack of fossil evidence, ARBER and PARKIN (2) suggested three possibilities as to the past history of the Gnetales: first, that they are aberrant survivals of a great and complex group dominant in the Tertiary vegetation; second, that they are a new group; third, that while in existence in the Tertiary they have always been a little varied and subsidiary element of the flora. The result of their study is the conclusion that they are nearest the Angiosperms of any of the several Gymnosperm lines, and that they are examples of parallel development from a common ancestor. This ancestor they would place among the Bennettitales, with the idea that Gnetalean floral organs have been derived from them by the reduction common to the evolution of an inflorescence. They cite *Populus glauca*, an Indian species described by HAINES, as an illustration of a similar process among Angiosperms, and one that furnishes an almost complete homology with *Welwitschia*.

JEFFREY (19) definitely opposed this idea, saying that the flower of *Welwitschia* offers no basis for comparison with a Bennettitalian cone, and that the similarity of the latter to an Angiosperm flower is entirely the superficial one of arrangement. He contrasts what he calls the epidermis-lined pollen chamber of *Ephedra* with the lysigenous one of lower Gymnosperms, and the Cycad fertilization by motile sperms with the siphonogamy of the Gnetales. In short, he holds that neither the Gnetales nor the Angiosperms are related to the Cycadophytes, but that there is no reasonable doubt of a Gymnosperm ancestry. This view is in accord with THOMPSON'S conclusions that the morphology of *Ephedra* affords no support for the idea of a Bennettitalian connection, and with the deductions

of BAILEY (4), BAILEY and TUPPER (7), and Miss BLISS (10) from their studies of vascular elements.

BERRY attributes to the Gnetales an ancient and collateral relationship with the other Coniferophytes, perhaps as far back as the Cordaitales region or the Cycadofilicales, and on the other hand through *Gnetum* with an unknown ancestor that may have produced the Angiosperms as well. In this connection it is interesting to recall the queries of CHAMBERLAIN (11) as to the possibilities of a lost herbaceous Gymnosperm flora that might have given rise to the herbaceous Angiosperms, that in turn produced the woody types of the Cretaceous; a theory that would obviate the otherwise evident need of deriving them from woody forms already known, the Cycadophytes or the Coniferales. There are some generalizations in WIELAND'S (31) paper also that may have point here. They are to the effect that there is a very small record of upland vegetation of past times, and that the flora of uplands and polar regions included the majority of plastic forms. The plants that were at once extinguished and preserved by subsidence made up the aplastic coastal floras.

WIELAND traces the Conifer line back through the Jurassic and Permian, the periods during which their inflorescences acquired the compact unisexual cone form, and connects them with the dominating Cordaitales of the Carboniferous. To Angiosperm alliances he finds no clue except the, to him, obvious one of the much varied Mesozoic Bennettitales. The slender forked stems of *Williamsoniella*, with their narrow pinnately veined leaves, and small bisporangiate flowers, in WIELAND'S opinion "demonstrate the presence of a great alliance related to the primitive magnolias and abundantly represented in the leafy records of the Mesozoic." The period of dominance of this great Cycadeoidean assemblage, extending as it does from the Paleozoic to the Cretaceous, was particularly fruitful in the evolution of the higher forms; and WIELAND points out that some East Andean representatives of the group were upland and scrub types, while one of them (*Dictyozamites*) even had netted veined leaves.

SCOTT (25) subscribes to this idea of Angiosperm derivation, and admits the possibility, in view of the indicated relationships between

Bennettitales and Gnetales (ARBER and PARKIN 2), of a future reference of the whole Gymnosperm division to that ancestral stock. He regards the Gymnosperms as monophyletic, an opinion which SEWARD has also recently expressed. A considerable discussion of the relative merits of a Lycopod and Cordaitalean connection for the Araucarieae, which he thinks are primitive, brings him to the conclusion that the points in common with the Cordaitales carry much the greater weight. He judges that the Araucarian cone scales are too complex for comparison with Lycopod sporophylls, and that there is little likeness between the ligules of the two groups. The decision is reached, therefore, that on the whole there is evidence sufficient to establish for the Araucarian tribe, and with them the other Gymnosperms, a real affinity for the Cordaitales, not however through the Cordaitaeae. For the Cordaitales in turn, despite the dissimilar habit and advanced character of their fructifications, there is a clear connection with the Cycadofilicales on the basis of seed structure and stem anatomy.

SINNOTT (26), using the leaf trace and foliar bundle characters as criteria, has erected a phylogenetic series that is especially interesting when compared with the alliances just described. It may be summarized as follows: The primitive foliar bundle, single, monarch and mesarch, characterizing the *Lycopsidea* is evidence of their primitive nature and uniformity. Its presence in the base of the leaf trace of *Osmunda* and some of the Ophioglossales indicates their early separation from the other ferns. The typical Paleozoic Filicales had a diarch, mesarch trace which has reached the endarch condition in the modern ferns by the disappearance of the centripetal wood. The derivation has been, apparently, through the ancient Botryopterideae. Furthermore, from forms related to the latter, but which had already acquired the seed habit, have descended the Spermatophytes along two independent lines. The first of these, represented by *Calamopitys*, *Lyginodendron*, and *Heterangium*, was characterized by fernlike habit, peculiar seeds, double leaf trace, and the association of the protoxylem with the centrifugal wood to produce ultimately the endarch stele. This line ended blindly. The other succession includes all the remaining Gymnosperms and probably the Angiosperms. Its early members showed less fernlike habit

(*Medullosa*), a higher type of seeds more like those of Cycads, a double leaf trace in most of the series, and a tendency for the protoxylem to become associated with the centripetal wood and to develop the exarch stele. From this exarch series developed two principal groups, the Coniferophytes, represented by Cordaitales, and the Cycadophytes, with the Bennettitales and Cycadales as members.

The salient points of this brief review of opinion may be summarized as follows. The Angiosperms are admitted to exhibit Gnetalean resemblances to the extent of warranting the supposition for them of a common ancestor. The Gnetales are accorded a place among the Gymnosperms, and these in turn are derived from the primitive seed plants through the Cordaitales. The Cycadales and Bennettitales represent a varied assemblage tracing its origin along another line to the Cycadofilicales. Contradiction is encountered in the efforts of some authorities to associate the Angiosperms and possibly the Gnetales with the early Cycadophyte series. From the Cycadofilicales the succession passes downward, in the opinion of some through the Pteridophytes; in that of others parallel with them, to the primitive vascular plants that exhibit traits of Algae, Bryophytes, and Pteridophytes and are of so simple and generalized a character as to qualify as progenitors for all the higher forms. With increasing knowledge, however, there is discernible a growing tendency to suspend judgment. We find WIELAND (31) observing that "The lengthening of the fossil record shows nearly universal parallelism instead of comparatively recent development of dichotomous stocks. Slowly converging lines replace the paleontologic tree" of which the branchings are rapidly being pushed back on anatomical grounds; and SCOTT (25) warns that "A more tentative and diffident tone seems to be demanded in discussing phylogenetic problems."

#### Primitive Angiosperms

Regarding the past history of this division of plants, two things may be said: that it is suprisingly short as plant records go, and inexplicably abrupt in its initiation. Records of Angiosperms of undoubted character and certain age have been assigned only as far back as the upper levels of the Lower Cretaceous. To the previous

evidence of leaf impressions Miss STOPES (28) has now added the surer testimony of structure to prove that these earliest known Angiosperms were not in any sense proangiosperms or primitive types. Both the Monocotyledons and the Dicotyledons appeared among the remains, and BERRY (8) calls attention to the fact that they were already an important element in the vegetation. They were practically all Archichlamydeae, but it is noteworthy that the epigynous *Eucalyphus* had appeared among them. By the close of the Upper Cretaceous most of the principal families were evolved, with the exception only of the highest; the orchids and the composites and Angiosperms had gone far toward attaining their present dominance. In Tertiary time the flowering plants were much modernized, and the Miocene floras differed from the present mainly in greater prevalence of the arborescent types. Referring to this "crowning achievement of plant evolution," BERRY says "one is almost tempted to see design in the world-wide radiation of flowering plants during the Upper Cretaceous times immediately preceding the age of mammals."

### Herbaceous Angiosperms

The further development of plant forms since the Miocene has resulted in the gradual usurpation of dominance by the herbaceous Angiosperms. This is the modern phase of which such groups as the Cruciferae, Labiatae, and Compositae are examples, and of which the history is apparently almost entirely post-glacial. SINNOTT (27) writes of the importance to man of this advance, in that it is closely correlated with the evolution of birds, insects, and mammals. The herbs as the subjects of primitive agriculture doubtless first enticed man from the forest, and have furnished the means by which he has wrought out his civilization.

The forces to which SINNOTT ascribed these changes resulted from disturbances of the uniformity of geological climates, the appearance of seasonal variation in temperature and moisture; that is to say, refrigeration and aridity. Among plants, then, the survivors were the very hardy woody types or the individuals so adapted as to hold over in the form of resistant seeds or hibernating underground stems. Organizing the evidence of floristics, general and

endemic, of distribution, fossil records, and plant anatomy, SINNOTT concludes that herbs are of recent and northern origin, a theory also expressed by WIELAND (32).

In comparison, there is no doubt of the antiquity of the woody forms and some of the older stocks, such as the *Equisetum* and *Lycopod* giants of the Carboniferous, are still represented by their degenerate descendants. In the whole curve of plant evolution, however, they stood at a point well up from the bottom. As to the character of the herbaceous plants that might have produced them, we are perhaps granted more than a glimpse in the discovery of the Devonian fossils; but these simple protostelic plants are separated by long periods of elaboration and modification from their dynamic progeny, the herbaceous vegetation of today.

The principal mechanical accompaniment of the evolution of herbs has been the breaking up of the siphonostele into a ring of bundles separated by parenchymatous tissue. To account for this, the most prominent hypothesis is that of the development of the rays; that is, the accumulation of storage tissue adjacent to the leaf traces as the result of greatly increased assimilative power, and their final disposition along the sides of the bundles as the vascular cylinder became progressively narrowed. When these bands of parenchyma extend from pith to cortex the stele consists of a ring of disconnected bundles, the typical herbaceous condition.

The possibility of using the pith ray systems in dicotyledonous woody stems, as indices of phylogenetic arrangement, has led to a great amount of investigation and to somewhat contradictory results. Among the various workers were THOMPSON (30), BAILEY (5), GROOM (15), Miss HOLDEN (17, 18), BAILEY and SINNOTT (6) who gave a very comprehensive report embracing fossil as well as living forms, Miss FLINT (13), and Miss LANGDON (23). Considerable interest attaches to the last named paper, in view of the conflicting results of the others. Miss LANGDON worked from a new approach, her object being to discover the possible modifying effects of age, location on the tree, and conditions of growth on the ray systems. The effects of the first two she regarded as negligible, but the ray systems were found to be so altered by differences in vigor of growth that she was led to consider them unreliable as indices of phylogeny.

For the polystele characteristics of Monocotyledons and of members of the Araliaceae and Umbelliferae a dicotyledonous origin is generally conceded. The great number of traces from the large parallel-veined leaves is commonly held accountable for the scattered arrangement of the bundles in the stem. But Miss SARGENT (24), writing in 1908, ascribed the characteristic differences in detail to the presence or absence of cambium. She used as illustrations certain Dicotyledons that have lost their cambium and developed a scattered arrangement and occasionally amphivasal structure. Miss SARGENT observes that cambium, while absent only in some Dicotyledons, is not really a functioning tissue in any of the Monocotyledons, although they still give evidence in their ontogeny and in some mature stem vestiges of its having been a factor in their phylogeny.

Another employment of dynamic tissues as an explanation of the disintegration of the siphonostele is made by LAND in his course in Spermatophyte morphology. He most interestingly demonstrates the performance of the pericycle in elaboration of the polystele from the unbroken dicotyledonous cylinder. The pericycle is the direct descendant of the primary meristem of the root and stem tips, and retains its potential activity, exercising it, in the presence of functional stelar cambium, only in the production of lateral members in roots.

The tendency to thickening of the pericycle cells of the stem causes, in many forms, the formation of patches of thick-walled cells capping the primary bundles. From this stage, LAND's material shows a series in the progressive encroachment of the pericycle bands upon the bundles, severing the connections of the interfascicular cambiums, finally forming a completely enveloping sheath and reducing the siphonostele to a ring of disconnected bundles, the first step in its disintegration. The process is closely correlated with the evolution of amphivasal structure. In the rhizome of *Acorus*, for example, can be found an excellent intermediate stage. The pith bundles are amphivasal and are inclosed in a ring of bundles retaining the siphonostele arrangement but exhibiting varying degrees of amphivasal development, while in the cortex the scattered traces are uniformly collateral.



With the passing of the cambium, to which LAND refers as a transitory tissue appearing late in geological history, the pericycle again becomes meristematic, and lays down a broad zone of cells among which appear strands of secondary desmogen that in turn give rise to the bundles of secondary growth. This is well illustrated in the stems of *Aloe pleuridens*.

The present evolutionary tendencies of plants are picturesquely summarized by LAND: "The polystele and the rhizome lie at the end of every phylum. Toward the attainment of this condition among Monocotyledons, for example, wheat is well on the way and the banana has arrived."

#### Present classification of Angiosperms

As expressive of the attitude of many botanists toward the ENGLER scheme of classification, the following words of BERRY (9) are quoted: "While very imperfect and founded to too great a degree on floral morphology, the classification proposed by ENGLER and derived largely from EICHLER's work is the most satisfactory and has the additional advantage of having been elaborated in a general systematic treatise"; but there is much opposition to the arrangement, especially as it applies to the primitive members of the Angiosperm group.

ARBER and PARKIN (2) bring together considerable evidence of reduction in the floral structure of Amentiferae which to them indicates derivation from higher forms. They hold with GOEBEL that the bisporangiate flower is primitive, and that plants with unisexual flowers represent a higher stage of development. They constructed a hypothetical primitive flower which they called the proanthostrobilus, and which was based on the bisporangiate Bennettitales cone described by WIELAND. From forms possessing flowers more or less like this they would derive the Angiosperms, with the Magnolias as the nearest living representatives. Thus they believe the Ranales to be the primitive members of this line. JEFFREY (19) sees little anatomical basis for this Bennettitales connection, and considers the question beyond solution without an acquaintance with the Angiosperms of the Jurassic or even earlier Mesozoic epochs.

COULTER and CHAMBERLAIN (12) express the opinion that the

primitive character of the bisporangiate flower is not a necessary assumption; that the majority of simple flowers should be regarded as primitive; and that this idea is in accord with the evidence of morphology and of history, as well as with the doctrine of evolution.

An extensive discussion of the subject, especially as it applies to the Fagaceae, may be found in HOAR'S (16) paper, with a review of the opinions of the investigators. A unique classification of the Centrospermae and allied families, based on F. MALLIGSON'S "sero-diagnostischen" researches, is graphically shown by KARSTEN (20); and it is interesting to note that, while the Amentiferae hold together fairly well, they are placed upon a branch originating in the region of the Berberidaceae.

In favor of the Amentiferae as primitive forms there is historical evidence of their great antiquity, the generalized condition of the archesporium, the prevalence of wind pollination, and the many admittedly primitive characters of *Casuarina*. Perhaps the most obvious deduction from a glimpse into the literature of the subject is that there is great need for continued morphological work among this heterogeneous assemblage.

With the hope that a detailed study of the organogeny of the flower in some of the earliest known of living Angiosperms might help in the solution of the problem, an investigation of *Sassafras variifolium*, *Betula alba*, *Alnus glutinosa*, and *Corylus americana* was begun. Only the results of the study of *Sassafras* will be presented at this time.

### Morphology of *Sassafras variifolium*

#### HISTORY AND DESCRIPTION

*Sassafras*, as a subject for investigation, is interesting from several standpoints. It is a monotypic genus of great antiquity, once widespread but now much restricted in range; it is a member of the great and varied order of Ranales, thought by many botanists to include the primitive living Angiosperms; and it has reached that stage of development represented by a distinctly unstable dioecism.

The fossil history of *Sassafras* in America is as long as that of any Angiosperm. It appears in the geological records in the Patapsco, the uppermost of the three Potomac formations of the Lower

Cretaceous (BERRY 8, 33). There it is associated with *Populus* and other early forms. COULTER and CHAMBERLAIN (12) mention *Sassafras* fourth in their list of Lower Cretaceous Angiosperms, giving chronological precedence to *Populus*, *Liriodendron*, and *Magnolia*.

According to BRITTON and BROWN (34), there is one species of *Sassafras* typical of eastern North America and another of Asia. The plant is described by them as follows: "A tree sometimes one hundred and twenty-five feet high, the trunk seven feet in maximum diameter, bark rough in irregular ridges, aromatic, the young shoots yellowish green, the twigs and leaves mucilaginous . . . , leaves oval, or entire becoming mitten-shaped, or three-lobed to about the middle." In regard to this last statement, BERRY (33) calls attention to the fact that some trees have the leaves regularly four- or five-lobed, while occasionally there are specimens with six lobes. In the Chicago region, where *Sassafras* figures mostly in the dune vegetation, it attains only a moderate height and would be ranked as a small tree or shrub.

### Materials and methods

Most of the material used grew in the vicinity of the southern shore of Lake Michigan, but some was obtained from northeastern Missouri. Dr. E. QUISUMBING, who had collected a variety of forms for work in connection with his studies on the stony layer in Gymnosperm seeds, very generously furnished *Sassafras* material already imbedded in paraffin, and this important assistance is gratefully acknowledged.

The collections extended over a period of ten months, from late October to late July, but the unavoidable length of some of the intervals has left some gaps in the sequence which it is hoped can be filled in the future. Very satisfactory results were obtained by the use of hot corrosive sublimate acetic in 70 per cent alcohol as a killing and fixing agent. Tissues that proved refractory when fixed with chromoacetic acid cut easily when prepared by this method. Haidenhain's iron haematoxylin and gold orange were the stains used for the most part, although some good preparations were made with alcoholic safranin and aqueous crystal violet.

## MORPHOLOGY OF FLOWER

The flower is described in Gray's manual as "dioecious or nearly so, with a six-parted calyx; the sterile kind with nine stamens inserted on the base of the calyx in three rows, the three inner with a pair of stalked glands at the base of each, anthers four-celled; fertile flowers with six short rudiments of stamens and an ovoid ovary. Drupe ovoid, supported on a club-shaped and rather fleshy reddish pedicel."

The ovary of the Lauraceae, to which *Sassafras* belongs, is one-celled; and in ENGLER's syllabus (36) the number of carpels is recorded as "3 (oder 1?)." On this question MIRANDE (37), after a study of several of the genera, stated that all show evidence of trimerous pistil formation, but that two of the carpels abort early and only one is continued into a style and stigma. The preparations of *Sassafras* studied for this investigation showed three fibrovascular bundles in the transverse sections of the upper part of the ovary, and a blind opening near the base of the style. The former would seem to indicate the trimerous condition in that part of the structure, and the latter may correspond to the "ovarian canal" of MIRANDE's description. In the absence of intermediate stages in the growth of the pistil, however, that phase of the floral development must be left for further investigation.

In the youngest available ovulate flowers, collected April 7, the pistil appears as a conical elevation at the bottom of a cuplike depression that bears on its rim the sepals and the rudiments of stamens (fig. 1). In the very young stage shown in fig. 2 there is little differentiation, except that of the epidermis and the large gland cells, so conspicuous a feature of the young tissues. In some of the flowers of this date were found slightly more advanced pistils showing the beginning of elongation (figs. 1, 3), but without any evidence of sporogenous tissue or carpel structure.

The next step encountered showed considerable advance, the ovary with style and stigma developed and the ovule already partially anatropous (fig. 4). A stout funiculus attaches the ovule well up on the ovule wall, and above this point a very narrow channel passes outward from the ovary cavity to the surface, doubtless indicating the line of closure of the carpel. The appearance at this time is

very similar to that of a young carpel of *Ranunculus*. Only one integument was discernible at this stage. In one case two large megaspore mother cells were found (fig. 5), one of them with the nucleus in synapsis, the other one with its nucleus in the resting stage. This ovule is particularly interesting because it occurred in a bisporangiate flower.

The material collected on May 5 showed the mature, 8-nucleate, typically angiospermous embryo sac. It is long and rather narrow, with deeply placed antipodals which are inconspicuous and evanescent, but with egg apparatus and fusion nuclei well developed. The pistil has at this time attained a length of about 4 mm., and the ovule itself, nearly a fourth of this, fills the ovary cavity. It is now completely anatropous, suspended by its overarching funiculus. The micropyle is formed by the beaklike development of the inner integument, that is compressed below into a narrow row of cells, which, however, is easily traceable on either side nearly to the base of the nucellus. The outer integument is more massive, but at this time shorter than the other. The nucellus is pointed at the micropylar end and projects well up into the opening. Periclinal divisions have produced five or six layers of cells between the embryo sac wall and the surface. The peripheral layers of the nucellar tissues are compact, but those surrounding the embryo sac are composed of large cells, thin-walled and delicate, that make the outlines of the embryo sac itself difficult to distinguish.

After fertilization, endosperm development is rapid, and a thin layer of cytoplasm with many free nuclei lines the embryo sac by the time of the first division of the egg. In the formation of the first wall of the proembryo, *Sassafras* seems to agree with *Leitneria* as described by Miss PFEIFFER (38). Its plane apparently is not fixed, since 2-celled embryos were found, showing that it may be either vertical or horizontal.

When wall formation begins in the endosperm those cells nearest the embryo are of moderate size; but the greater part of this tissue is composed of extremely large, thin-walled cells which are conspicuous at as late a stage as that shown in fig. 19.

There is further agreement with *Leitneria* in the character of the cells that produce the flat-topped proembryo and the massive

embryo with its short, wide suspensor. The development during May is shown by figs. 11-16. Fig. 17 represents the condition reached early in June: a large mass of cells still without definite indication of the cotyledons. The cells of this embryo had a peculiar appearance due to extreme vacuolization, a detail of which is given in fig. 18.

The cells of the nucellus form a loose thin-walled perisperm about the embryo sac; and the ovary cavity, which now measures about 6 mm. in length, appears when cut open to be filled with a glistening transparent jelly. The peculiarly shaped embryo, with its wide-spread cotyledons, occupies but a portion of this space, since it measures only 1.3 mm. in width and scarcely 0.5 mm. in length. The cotyledons develop winglike lobes (figs. 19, 20) that extend beyond the hypocotyl and completely inclose it. The body of the embryo is still massive. In the most advanced stage examined, one taken from a ripening fruit, the stem tip had retained its broad top and was capped by the curving tips of the first leaves (fig. 20). The cotyledons at this time filled the whole interior of the ovule, measuring nearly 7 mm. in length, and had absorbed all the available food supply, their cells being packed with starch. The perisperm was reduced to a thin enveloping skin. Well developed spiral vessels were evident in the axis and in the fibrovascular bundles of the cotyledons.

#### STONY AND TRANSFUSION LAYERS

The behavior of the tissues surrounding the nucellus is interesting. QUISUMBING (39) has briefly described the development of the stony layer in *Sassafras* as a palisade growth of the epidermal lining of the ovary. The elongation of these cells about the micropylar region of the ovule may be seen in fig. 8. By the time the embryo has reached the condition indicated in fig. 19, these cells have become very prominent, long, thick-walled, and pitted; but in the sections cut at right angles to the faces of the cotyledons, and therefore across the prominent ridge that is formed around the upper portion of the stony coat, the differentiation ends abruptly about a third of the way down from the micropyle. The lower cells of this layer are at this time oblong in section and show no thickening of the lateral walls. In the ovule itself considerable transfusion tissue shows at this period, developing apparently in the outer integument.

About the apex of the nucellus this is several layers thick, and composed of large cells, but it is reduced below to one or two layers of narrowly oblong cells.

When the fruits containing embryos of the largest size figured were cut, the whole inner layer of the ovary wall was hard, although perceptibly less resistant in the region of the pedicel. Its development, then, seems not to be of uniform progression, but the later stages of modification must take place rather rapidly with the ripening of the seed.

#### STAMINATE FLOWER

The staminate flower presents an interesting topography on account of the 4-chambered stamens with their valves, and the conspicuous paired glands at the bases of the inner row of filaments. In the center of the normal flower there is a rudimentary pistil very similar to that represented in fig. 2, although its cells appear to be even nearer active development.

The staminate glands are reniform in longitudinal section in the plane of their greater width. They have a rich vascular supply, spiral vessels spreading fanwise from the stalk and frequently ending in direct contact with the large gland cells. The latter, characteristic of all the tissues, are particularly noticeable here because of their number. They first show as somewhat enlarged cells with homogeneous cytoplasm and a central nucleus. Then vacuolization begins and increases until the cell content is crowded into a thin peripheral layer with a shrunken and flattened nucleus. Eventually the protoplasm disappears and the cell, several times its original size, becomes filled with the characteristic fluid. In sections from older flowers, especially in the region of the thickened pedicel, canals occur that apparently have been formed by the breakdown of contiguous cells of this description.

#### MICROSPORANGIUM

In stamens of flowers gathered in November there were found microspore mother cells in prophase and others in anaphase of the heterotypic division, while many anthers contained fully formed microspores. The tapetum in all these stages is very conspicuous. It consists of large, deeply staining, often binucleate cells that begin to disintegrate before the microspores are formed, so that the young

pollen grains lie in the midst of a plasmodium with many flattened nuclei. Remnants of this plasmodium are still evident in the mature sporangia of flowers collected just before anthesis in the spring. The microspores give early evidence of abundant nourishment, and when mature are packed full of large starch grains (fig. 24). The exine is beset with short straight spines and the spores are shed in the binucleate stage. Flowers brought in May 10, 1923, were shedding pollen, but the season was nearly a month later than the previous one.

The opening of the anthers, which in *Sassafras* is by the rather spectacular arrangement of four uplifted valves, is accomplished by the development of a peculiar endothecium. The cells below the slightly rounded ones of the epidermis become elongated at right angles to the axis of the anther, and then are heavily thickened (fig. 24). A deep groove marks the dehiscence line along the sides and bottom of this area of heavy cells, but it does not appear at the top where the cells are especially conspicuous for their size.

#### BISPORANGIATE FLOWER

A number of bisporangiate flowers were found, in every case with fertile stamens but with the pistils in various stages of development. Some of these were obviously merely cases of delayed abortion; but one, from which fig. 5 was made, had developed up to the megaspore mother cell stage with every sign of continuing. This specimen was from a collection made in November, and the appearance of its pistil is in marked contrast with that of the one shown in fig. 1, belonging to a supposedly normal ovulate flower, collected April 7 the following spring. This latter material was scanty, however, and it is possible that it may not have represented normal development of the pistil of that date. It will be interesting to discover whether or not that is the case, and also whether or not the bisporangiate flowers develop seeds.

#### Discussion

Inasmuch as *Sassafras* is the single survivor of an ancient race, of which much of the history is known, and is placed taxonomically in an order classed as genetic and primitive because of the generalized condition of many of its members, any primitive or distinctive characters in its morphology are worthy of note.



While the evidence of a possible reduction in the pistil and the anatropous character of the ovule indicate the accuracy of its classification as near the apex of the Ranales group, the formation of more than one archesporial cell in the nucellus, with indications that this tissue may be even more extensive, may be regarded as a primitive feature. Aligned with this also is the relatively massive nucellus. The common occurrence of the dioecious condition in the floral organization implies a history long enough to have permitted its evolution from the monoecious type; but its instability indicates a degree of development below that of many Angiosperms of more recent origin. In this respect, *Sassafras* falls within the number described by COULTER (35) as follows: "Many of the dioecious Angiosperms, however, seem rather recently to have been derived from ancestors which have two sexes represented in the same flower (or at least on the same plant). In these the dioecious condition seems not to have been firmly established; a regular sex chromosome mechanism has not yet been perfected," and is therefore much more subject to fluctuation under environmental influence than the long established unisexual forms.

Leaves of the lobed type like those of *Sassafras* doubtless represent modifications of an originally simple leaf, and this again signifies that much time has elapsed in the process of development. This genus, as already mentioned, is among the oldest of the Angiosperms known, but the earliest fossil records show that the Lower Cretaceous species of *Sassafras* had bilobed and trilobed leaves. With this feature carried so far, the possible character of the flower becomes an interesting subject for speculation. There might be pictured at least so primitive a condition as a bisporangiate flower, with three separate carpels on a common stalk, each bearing a single orthotropous ovule. From this it is not difficult to visualize the gradual abortion of the two carpels, and the development downward of the remaining one in such a way as to cause the suspension of the ovule from the upper part of the ovary wall and the ultimate assumption of the anatropous position.

Another point of interest from the standpoint of survival value is the evidence throughout the plant of high assimilative power and effective nutrition. Cross-sections of growing twigs disclose a pith laden with starch granules. The vascular supply to the floral organs

is conspicuous in all of the sections; the secretory organs are strikingly well developed; the microsporangia have a prominent tapetum and are later supplied with a plasmodium; the microspores are crowded with starch grains; the cotyledons are large and well supplied with food; and the seed is protected by a hard coat that appears to be remarkably resistant for the amount of stony tissue developed.

### Summary

1. The one-celled ovary of *Sassafras* is normally borne in an ovulate flower in which there are 6 rudimentary stamens. The ovule is solitary, anatropous, and may form more than one archesporial cell. The nucellus is massive and has two integuments.

2. The embryo sac is of the typical 8-nucleate type, but the antipodals are small and evanescent. In the endosperm many free nuclei are formed before wall formation begins. The cells are small near the embryo but very large in the middle of the sac. The perisperm is conspicuous until a late stage.

3. The first division of the fertilized egg occurs when the free nuclear stage in the endosperm is nearly completed, and may be either longitudinal or transverse.

4. The embryo is massive, with short suspensor and peculiarly auricled cotyledons. It eventually fills the ovary cavity. The seed is without endosperm.

5. A stony layer is developed by the elongation and thickening of the innermost layer of the ovary wall. Transfusion tissue forms in the outer integument, noticeably in the micropylar region.

6. The microspores are formed in most of the sporangia by November. A plasmodium of tapetal origin is conspicuous. The microspores are binucleate at time of shedding.

7. Bisporangiate flowers are not uncommon, and show ovulate development apparently much in advance of the normal fertile flower.

8. While *Sassafras* is one of the earliest known Angiosperms, its Mesozoic records do not differ much from the corresponding features in the living genus which indicates a much more ancient, unrecorded ancestry.

The writer desires to express sincere appreciation of the constant guidance of Professor C. J. CHAMBERLAIN, under whose direction

the work was done; and of the inspiration and encouragement of the other members of the botanical staff of the University of Chicago.

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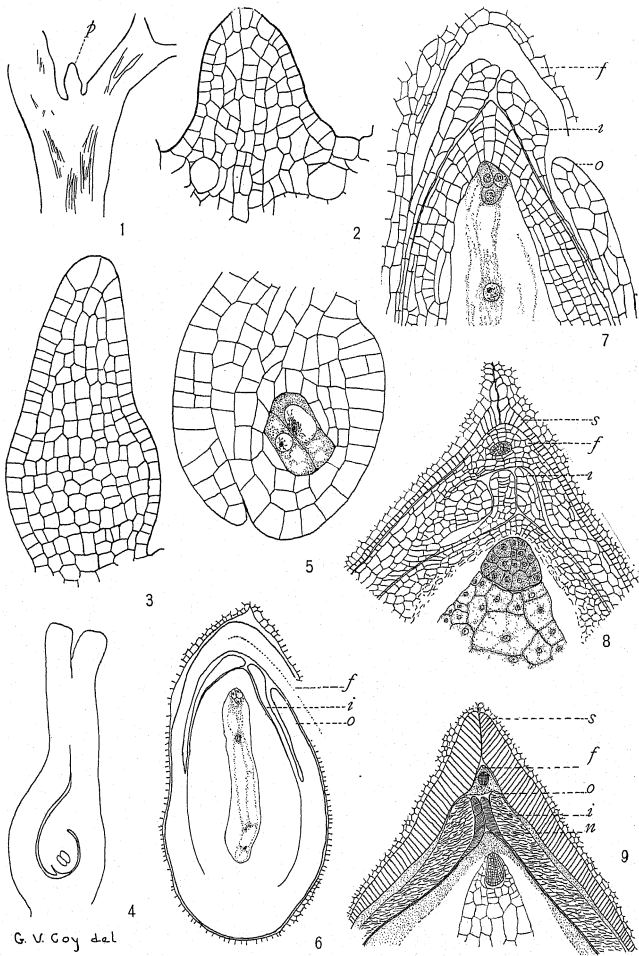
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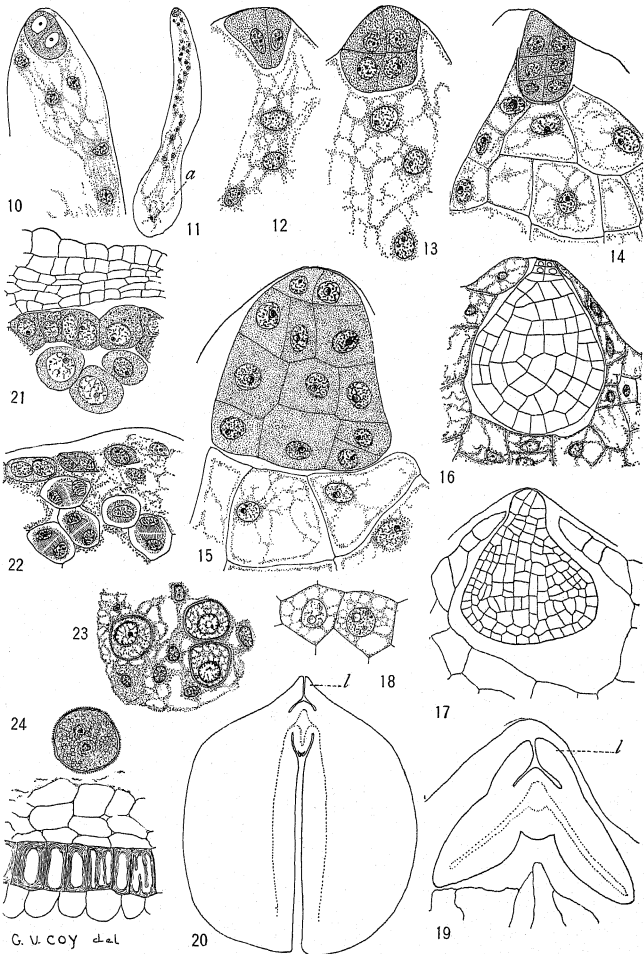
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## EXPLANATION OF PLATES VIII, IX

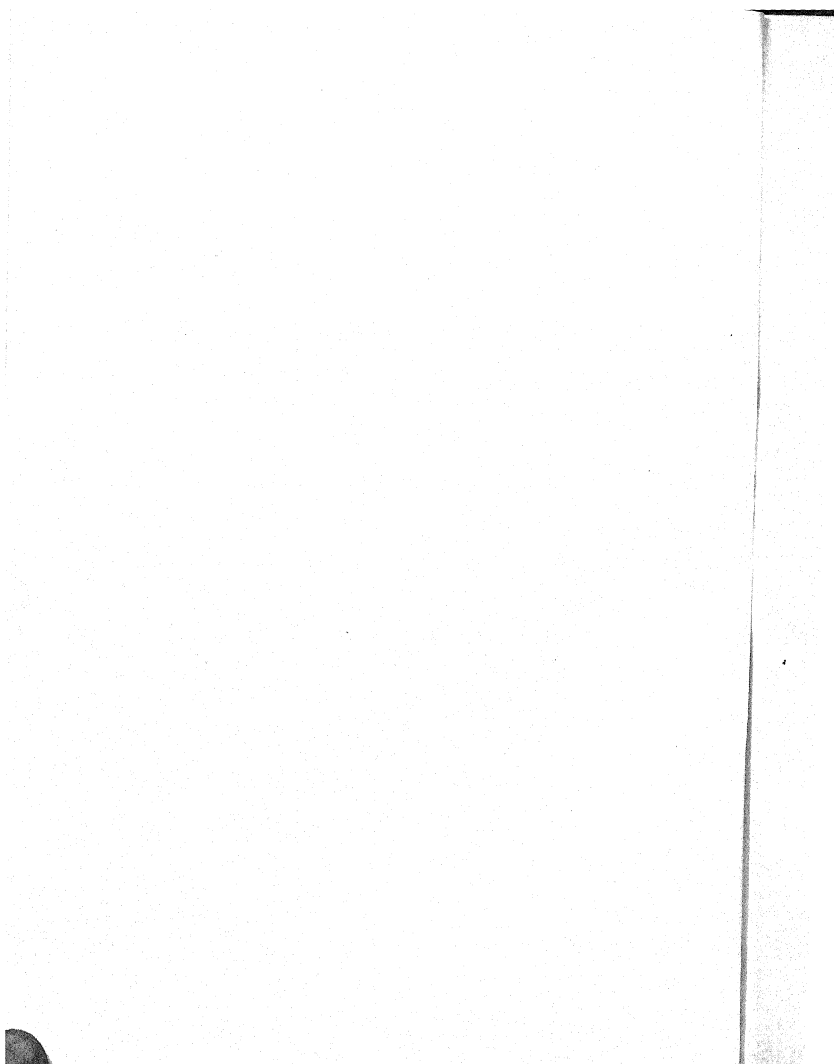
All drawings were made with the aid of the Abbé camera lucida. The abbreviations are as follows: *a*, antipodals; *f*, funiculus; *i*, inner integument; *l*, lobe of auricled cotyledon; *n*, nucellus; *o*, outer integument; *p*, pistil; *s*, stony layer.







G. V. COY del.





## PLATE VIII

FIG. 1.—Longitudinal section of young ovulate flower showing pistil;  $\times 25$ .

FIG. 2.—Enlargement of pistil somewhat younger than that in fig. 1, two gland cells visible on either side near base;  $\times 210$ .

FIG. 3.—Enlargement of pistil shown in fig. 1;  $\times 210$ .

FIG. 4.—Diagram of longitudinal section of pistil from bisporangiate flower of November 26, showing ovule and archesporial cells;  $\times 80$ .

FIG. 5.—Enlargement of ovule from fig. 4, showing detail of archesporial cells;  $\times 475$ .

FIG. 6.—Diagram of ovule with embryo sac before fertilization, showing position of funiculus and integuments, egg apparatus, fusion nucleus, and antipodals;  $\times 80$ .

FIG. 7.—Enlargement of micropylar region of ovule shown in fig. 6;  $\times 210$ .

FIG. 8.—Micropylar region of ovule with proembryo, portion of funiculus, and elongating cells of ovary wall that will form the stony layer;  $\times 80$ .

FIG. 9.—Diagram of same region when embryo has reached stage shown in fig. 17, showing elongation of cells of stony layer and transfusion tissue in outer integument;  $\times 50$ .

## PLATE IX

FIG. 10.—First division of fertilized egg by horizontal wall; free nuclei in endosperm;  $\times 350$ .

FIG. 11.—Topography of embryo sac at this stage;  $\times 50$ .

FIG. 12.—First division of egg by longitudinal wall;  $\times 475$ .

FIG. 13.—Second division of proembryo;  $\times 475$ .

FIG. 14.—Proembryo showing further division and endosperm with walls formed;  $\times 475$ .

FIG. 15.—Beginning of massive development of proembryo;  $\times 475$ .

FIG. 16.—Embryo of many cells in globular mass, with short suspensor;  $\times 350$ .

FIG. 17.—Embryo ten days older than that in fig. 16;  $\times 210$ .

FIG. 18.—Detail of vacuolated cells of embryo shown in fig. 17;  $\times 650$ .

FIG. 19.—Embryo showing well developed, auricled cotyledons;  $\times 210$ .

FIG. 20.—Embryo extending full length of ovule and showing very large cotyledons and very small first leaves;  $\times 10$ .

FIG. 21.—Portion of microsporangium showing wall cells, tapetum, and microspore mother cells;  $\times 475$ .

FIG. 22.—Portion of microsporangium with spore mother cells completing heterotypic division; tapetal cells partially broken down and forming plasmodium;  $\times 475$ .

FIG. 23.—Young uninucleate microspores surrounded by plasmodium;  $\times 475$ .

FIG. 24.—Portion of microsporangium showing endothecium, inner wall layers; and microspore with tube and generative nuclei, and numerous starch grains;  $\times 475$ .

## YELLOWS OR LITTLE-LEAF OF WALNUT TREES<sup>1</sup>

A. R. C. HAAS, L. D. BATCHELOR, AND E. E. THOMAS

(WITH FIVE FIGURES)

Yellows or little-leaf of the walnut is characterized by the growth of small, yellowish green foliage, which frequently serves to distinguish the disease at a considerable distance. The twig growth

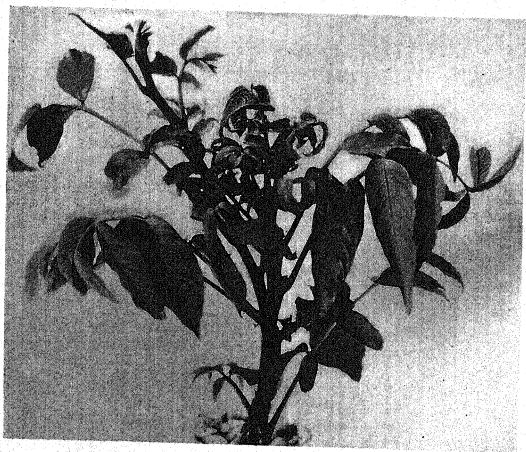


FIG. 1.—Terminal twig growth of walnut affected with yellows

usually has extremely short internodes, with the compound leaves closely clustered together; and oftentimes the actual size of the leaflets is reduced to one-tenth or less of the normal size. The dorsal surface of the petiole of the compound leaf is curved convexly. The leaflets are commonly curved in a similar manner, and also tend to

<sup>1</sup> Paper no. 188, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

close along the midrib on the dorsal surface. This is well illustrated in fig. 1, which shows typical diseased terminal growth. The leaflets may have a yellowish green color, resembling that of chlorosis, or may show various degrees of mottling (fig. 2).

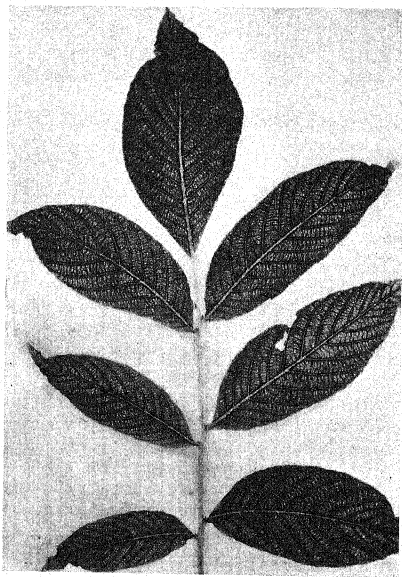


FIG. 2.—Walnut leaflets moderately affected with yellows, showing mottling

Marginal burning of the foliage is not necessarily typical of this disease, but may be present as a result of excessive salt content of the soil solution or other toxic conditions.

The root system of the diseased trees is characterized by a brownish, unhealthy appearance and a lack of fibrous rootlets.

In the advanced stages of the disease there may be an actual

dying back of the terminal growth. In extreme cases the disease has been known to kill entire trees, regardless of their age. Young trees from one to ten years of age have been known to recover from yellows without having received any remedial treatment; conversely, trees which have maintained a normal growth for a period of ten to fifteen years have been known to develop the disease gradually, even to the point of ultimate destruction.

SMITH ET AL. (7) maintain that walnut yellows is associated with abnormally long dry seasons, or any other soil or irrigation conditions which may bring about a prolonged abnormal dryness of the subsoil. HODGSON (1) attributed winter injury of walnut trees to nematodes when it was characterized by foliage reduced in size and distinctly paler than normal in color.

Walnut yellows seems to manifest certain symptoms in common with pecan rosette (fig. 3), mottling of citrus, and the little-leaf of apricot and peach (fig. 4). ORTON and RAND (5) believe that pecan rosette is nonparasitic and is not transmissible. They observed the disease on many different types of soils and under various cultural conditions, but were unable by means of ash analyses to show any difference in composition between diseased and normal trees. They state, however, that the disease is caused directly or indirectly by some soil relationship which brings about a lack of balance in the plant-food materials or some unfavorable physical condition, although no definite evidence is offered to substantiate this statement.

RAND (6) views pecan rosette from a pathological standpoint, and gives a very extensive review of the literature. McMURRAN (4) believes that a deficiency of humus, fertility, and moisture supply is a causal factor in producing pecan rosette. McCLINTOCK (3) was unable to obtain transmission of peach rosette by means of soil, or of sap from diseased trees. Likewise the insect transfer of the disease was considered improbable; nevertheless this author concludes that peach rosette may be transmitted from one tree to another "by means of buds."

Walnut yellows is widely distributed in southern and central California. Practically every walnut-growing district in the state has areas affected with this disease, regardless of climatic conditions. The soil types on which the disease most frequently occurs are as

follows: Hanford fine sandy loam, Placentia loam, Ramona loam, and Yolo clay loam.



FIG. 3.—Terminal twig growth of pecan affected with rosette

The use of irrigation waters with various amounts and kinds of impurities does not bear any direct relationship to the occurrence of this disease. Diseased trees are found growing in groves which

receive all extremes of cultural and irrigation conditions. These conditions range from dry farming to over-irrigation, and from clean culture to growth in sod or (dooryard trees) in driveways. No one type of walnut rootstock or variety of walnut seems more susceptible



FIG. 4.—Peach twig affected with little-leaf, compared with twig bearing normal leaves.

to the disease than any other. Our observations lead us to believe that there is a direct relationship between various tree troubles such as walnut yellows, pecan rosette, citrus mottling, peach little-leaf, and apricot little-leaf.

A study has been made of the occurrence of nematode (*Heterodera radiculicola*) upon the roots of healthy and diseased walnut trees.

Not only the roots of diseased but also those of healthy trees were frequently found to be infested with the nematode; conversely, the roots of diseased and of healthy trees were frequently found to be free from nematode attack. It is obvious, therefore, that the presence of nematode infestation cannot be related to the occurrence of the disease.

Some investigators attribute the occurrence of these diseases to a low nitrogen content in the soil. No study seems to have been made regarding the effect of soluble salts upon the prevalence of these diseases. Table I shows the results obtained for analyses of 1:5 water extracts of soil from adjacent healthy and diseased groves. These results are typical of many such comparisons. Only the anion content of the water extracts was determined. The carbonate, bicarbonate, chlorine, nitrate, and sulphate contents are given in terms of parts per million of dry soil. A comparison of the concentration of the anions in the water extract of the soils shows no important differences between the soils from healthy and those from diseased groves. Our data lead us to believe that these diseases are independent of the nitrogen content of the soil, although this is contrary to the conclusion drawn by several previous investigators.

Previous workers have failed to consider the influence of base exchange upon the nutrition of the trees. Usually the base exchange phenomenon has not been considered when nitrogen has been applied to the soil. For example, when nitrate of soda is added to the soil, a considerable part of the sodium goes to replace the calcium and other bases, and as a consequence the soil solution is richer in sodium and nitrate but also is greatly enriched with calcium and other bases. As this type of fertilization is continued, a soil solution may become depleted by leaching of much of the soluble calcium and other constituents, and finally enriched in sodium. Also alkalinity may develop in consequence of these soil changes, and this may finally reduce the solubility of the calcium, iron, etc., in extreme cases. Deflocculation of the soil may also result from such base exchange. In the investigations herein reported these complicating factors of nitrate fertilization have been considered.

At the outset of the investigation healthy and diseased walnut leaves were collected from different localities, from such extreme

TABLE I  
ANALYSES OF 1:5 WATER EXTRACTS OF SOIL FROM ADJACENT HEALTHY AND DISEASED WALNUT GROVES

DEPTH OF SAMPLE FROM SURFACE OF SOIL (FEET)	PARTS PER MILLION OF DRY SOIL											
	CO <sub>2</sub>		HCO <sub>3</sub>		Cl		NO <sub>3</sub>		SO <sub>4</sub>		Total solids	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Comparison I: adjacent orchards, Placencia loan												
1.....	0	0	92	107	27	27	4	6	Trace	Trace	210	210
2.....	0	0	107	107	18	18	Trace	Trace	Trace	Trace	160	220
3.....	0	0	122	122	27	35	Trace	Trace	Trace	Trace	185	170
4.....	0	0	122	107	18	18	Trace	Trace	Trace	Trace	165	130
5.....	0	0	107	107	35	18	Trace	Trace	Trace	Trace	105	200
6.....	0	0	107	107	27	27	0	0	Trace	Trace	100	185
7.....	0	0	107	107	35	35	0	0	Trace	Trace	160	270
8.....	0	0	183	198	27	27	0	0	Trace	Trace	165	265
9.....	0	0	183	183	27	27	0	0	Trace	Trace	200	265
10.....	0	0	108	213	27	27	0	0	Trace	Trace	200	275
11.....	0	0	167	183	18	27	0	0	Trace	Trace	200	275
12.....	0	0	183	183	18	18	0	0	Trace	Trace	185	120
Comparison II: adjacent orchards, San Joaquin loan												
1.....	0	0	107	30	27	27	12	13	Trace	Trace	200	200
2.....	0	0	107	76	27	35	10	15	Trace	Trace	200	215
3.....	0	0	107	92	27	44	5	8	Trace	Trace	205	220
4.....	0	0	137	122	27	35	6	9	Trace	Trace	210	210
5.....	0	0	107	122	18	53	4	26	Trace	Trace	170	235
Comparison III: adjacent orchards, Yolo clay loan												
1.....	0	0	107	107	44	44	53	53	Trace	Trace	445	460
2.....	0	0	92	92	53	35	13	40	Trace	Trace	195	255
3.....	0	0	107	92	71	44	7	12	Trace	Trace	185	225
4.....	0	0	76	92	27	35	13	6	Trace	Trace	290	215
5.....	0	0	107	92	35	27	Trace	7	Trace	Trace	185	235



conditions of tree growth as are illustrated in fig. 5. Analyses of the ash of such leaves gave very conflicting results, due to the fact that the leaves of the two types differed in age. This factor may account for the inconclusive results of many previous investigators in regard to the effect of the disease upon the composition of the

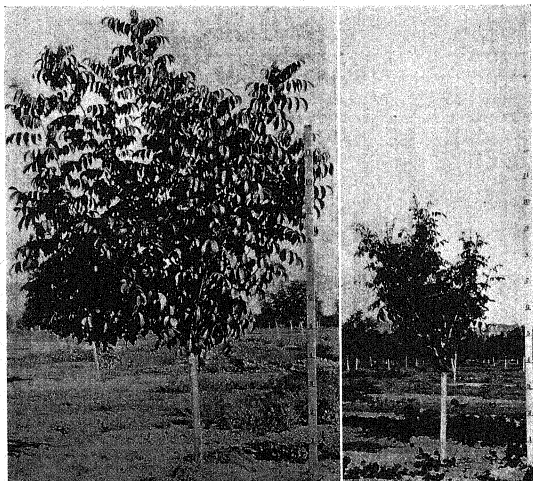


FIG. 5.—Extreme conditions of walnut tree growth in absence and presence of yellows.

leaves. After a great amount of preliminary work, it was finally decided to make first a study of the effect of age upon the composition of healthy and diseased walnut leaves.

The writers were fortunate in having available healthy and diseased trees growing upon the Experiment Station property. The past history of the occurrence of the disease upon the trees in question was well known, certain trees having been consistently normal, and other trees extremely diseased over a period of years. As new

shoots appeared on each class of trees, one could be quite certain of the ultimate nature of the resulting growth.

When the leaves on the two extreme types of trees were about one month old, tags were placed about the leaf stalks in order to identify leaves of the same age. At intervals of from five to seven weeks, samples of leaves of the same age were collected from healthy and diseased trees. These were dried at 70° C. and the composition of the ash was determined in the usual manner. The results are tabulated in table II. A study of the data of the trees on the Rubidoux tract shows a very rapid increase in ash content at the outset of growth, and later a slow but progressive increase until maturity is reached, after which there may be a slight decrease. By July 1 the ash content of the dry matter of the leaves is about three-fourths of that at maturity.

With the increase in the age of the leaves there is a decrease in the percentage of inorganic phosphate in the ash. The percentage of magnesium, chlorine, and sulphate in the ash remains relatively constant throughout the growth of the leaves. A matter of particular interest is the relation between the percentages of sodium and potassium in the ash, which decrease with increasing age of the leaves; and that of calcium, which is greatly increased as the leaf approaches maturity. It is of interest to note in this connection that the inverse relationship between the sodium and potassium percentages and the calcium percentage in the ash is quite analogous to that which exists in the ash of citrus leaves, as shown by KELLEY and CUMMINS (2). The calcium of normal mature walnut leaves usually ranges between 17 and 20 per cent of the ash.

When comparisons are made between healthy and diseased leaves of the same age, it is found that the percentage of potassium in the ash of the former is consistently lower than in that of the latter. A similar comparison shows that the percentage of calcium is consistently higher in the ash of healthy leaves than in that of diseased leaves. In this regard the ash of diseased leaves has the same composition as that of less mature normal leaves. This is precisely the same relationship that KELLEY and CUMMINS found to exist in the ash of healthy and mottled citrus leaves.

With this information with regard to the effect of the age of the

TABLE II  
COMPOSITION OF ASH OF DRY MATTER OF HEALTHY AND DISEASED WALNUT LEAVES IN RELATION TO AGE

	TREES FROM RUBIDOUX TRACT							TREES FROM BOX SPRINGS TRACT											
	Healthy							Dis- eased	Heal- thy	Dis- eased	Heal- thy	Dis- eased	Heal- thy	Dis- eased	Heal- thy	Dis- eased	Heal- thy		
Date of sam- pling.....	5/6	6/6	6/17	7/31	9/28	10/20	11/10	5/7	.....	6/23	6/23	8/5	8/5	9/28	9/28	10/20	10/20	11/10	11/10
Ash (as per- centage dry matter)....	6.46	9.01	10.33	10.99	12.79	12.90	11.81	6.65	.....	10.16	9.71	11.22	10.76	12.57	11.88	13.05	9.95	13.92	11.77
Ash constituents (percentage of ash)																			
Na.....	6.45	5.96	5.53	4.50	3.51	2.41	3.46	5.91	.....	3.03	4.84	1.82	2.26	1.27	2.36	0.53	2.34	1.10	0.65
K.....	26.16	24.63	20.36	16.60	13.15	12.00	13.08	20.42	.....	12.58	17.43	7.17	7.68	5.36	7.62	5.83	7.60	4.52	5.59
Ca.....	13.07	16.40	18.54	22.59	24.33	25.53	24.72	3.20	.....	24.46	17.07	26.02	22.57	27.19	21.78	28.39	25.62	29.02	27.31
Mg.....	5.07	4.31	4.59	4.41	4.64	4.60	4.77	6.45	.....	6.50	7.08	6.74	7.99	7.34	7.51	6.96	9.59	6.85	9.02
Cl.....	0.88	0.74	0.79	0.81	0.85	0.91	1.10	1.20	.....	1.64	3.18	1.20	2.78	0.90	2.02	0.94	3.49	1.11	3.52
SO <sub>4</sub> .....	4.08	3.44	3.13	3.69	4.49	4.01	4.25	4.08	.....	3.54	4.59	3.39	4.27	3.27	3.83	3.50	4.63	2.74	4.06
PO <sub>4</sub> .....	15.93	11.28	8.47	6.06	5.95	6.08	5.96	13.72	.....	6.38	8.93	4.48	7.30	5.19	6.00	4.45	5.70	4.68	3.59

leaf upon the composition of the ash, additional samples of leaves of known similar age were collected from healthy and diseased trees in several localities. The data reported in table III further justify the conclusion, drawn from table II, that the ash of diseased leaves is higher in potassium and lower in calcium than leaves from healthy trees.

A comparison of the ash constituents of similar portions of healthy and diseased walnut trees is presented in table IV. The analytical data from the various portions of the compound walnut

TABLE III  
COMPOSITION OF ASH OF DRY MATTER OF HEALTHY AND DISEASED  
WALNUT LEAVES OF SAME AGE

	COMPARISON I CHINO		COMPARISON II ARLINGTON		COMPARISON III ARLINGTON		COMPARISON IV ANAHEIM	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Ash (as percent- age dry mat- ter).....	10.56	9.73	10.48	9.61	10.73	11.55	12.15	14.50
Ash constituents (percentage of ash)								
Na.....	4.80	6.10	3.48	5.25	4.83	4.92	4.53	6.08
K.....	20.34	24.27	12.79	23.06	18.31	19.89	17.62	21.02
Ca.....	18.37	14.80	22.13	13.90	17.48	13.87	20.83	17.87
Mg.....	2.60	2.52	5.83	5.68	5.77	6.76	5.88	4.76
Cl.....	1.44	1.86					4.52	3.18
SO <sub>4</sub> .....	4.30	3.57	2.63	4.65	3.14	3.50	2.39	3.10
PO <sub>4</sub> .....	5.53	6.67	13.02	12.66	15.15	17.58	12.01	8.15

leaves are comparable with those obtained from the complete leaves. The inverse relationship between the percentages of calcium and potassium in the ash of healthy and diseased leaves is further confirmed. The same conclusion may also be drawn from the ash analyses of the husks and the matured twigs of the last growth cycle. The differences obtained, however, may not be significant. Nearly half of the ash of the walnut husk is potassium.

The ashes of mottled citrus leaves and of walnut leaves affected with yellows are related because their ashes contain a higher percentage of potassium and a lower percentage of calcium than that of normal leaves. This same relationship is shown by table V to

TABLE IV  
COMPARISON OF ASH OF DRY MATTER OF SIMILAR PORTIONS OF HEALTHY AND DISEASED WALNUT TREES

Ash (as per- centage dry matter)	LEAVES		LEAFLETS		LEAFSTALKS		COMPLETE COMPOUND LEAF		MATURED LAST CYCLE OF TWIGS		HUSKS		KERNELS	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
14.39	11.04	11.89	10.18	19.40	14.66	12.71	11.53	5.94	7.71	23.53	22.51	1.74	1.83	
Ash constituents (percentage of ash)														
Na.....	1.35	3.93	2.78	3.66	1.82	3.64	2.02	4.08	1.31	0.92	10.30	12.89	7.13	5.76
K.....	9.41	14.07	10.41	18.49	5.98	13.22	8.18	17.23	5.28	6.04	42.61*	42.82†	21.29	23.27
Ca.....	28.19	22.54	24.04	16.34	30.84	23.76	26.93	18.07	32.53	29.98	6.44	5.89	4.91	5.03
Mg.....	4.52	3.42	4.84	4.26	4.90	6.72	5.08	5.06	3.99	4.08	1.20	0.86	1.50	1.72
Cl.....	1.05	1.80	1.64	2.01	0.50	0.58	1.29	1.97	0.15	0.26	0.63	1.18	0.37	0.52
SO <sub>4</sub> .....	3.06	4.57	1.56	2.37	7.93	6.40	2.36	3.61	0.90	1.90	2.00	3.11	.....	.....
PO <sub>4</sub> .....	4.60	16.39	3.66	4.57	1.49	1.88	3.15	4.07	10.71	15.28	3.54	7.64	.....	.....

\* Equals 10.03 per cent of dry matter.

† Equals 9.64 per cent of dry matter.

apply equally well to leaves from pecan trees affected with rosette, and grape leaves affected with mottling and little-leaf, when compared with normal leaves of the same age.

In consideration of the abnormally low calcium and high potassium percentage in the ash of diseased walnut leaves as compared with healthy leaves, it is of interest to determine the water solubility of the inorganic constituents of the dry matter at increasing ages of the leaves. Table VI shows the relatively constant values obtained for the ash of the water-soluble fraction of walnut leaves of

TABLE V  
COMPOSITION OF ASH OF DRY MATTER OF HEALTHY AND DISEASED  
PECAN AND GRAPE LEAVES

	PECAN								GRAPE	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Ash (as percentage dry matter)										
	9.42	8.21	8.65	8.69	8.43	7.45	10.13	8.06	5.26	9.90
Ash constituents (percentage of ash)										
Na.....	2.74	4.13	3.35	4.17	3.52	4.16	3.84	4.41	5.21	9.02
K.....	11.37	16.72	10.59	13.97	13.16	15.82	12.36	17.70	21.28	31.29
Ca.....	22.33	19.27	23.51	19.91	21.58	19.77	22.95	17.76	13.42	11.13
Mg.....	8.21	7.03	7.92	6.94	8.44	8.20	5.61	6.99	6.86	2.74
Cl.....	0.83	0.91	0.74	0.82	0.33	0.80	0.32	0.84	0.84	0.91
SO <sub>4</sub> .....	2.77	3.16	3.12	3.03	2.88	3.48	3.74	3.44	3.56	3.51
PO <sub>4</sub> .....	7.15	9.52	6.86	8.94	6.73	10.54	6.70	10.85	20.12	44.70

varying ages, both healthy and diseased. The ash of the water-insoluble fraction shows considerable increase, however, with an increase in the age of the leaves. The data further show that the percentage of calcium in the ash of the water-soluble fraction of the dry matter of healthy leaves makes consistent but extremely small increases with increasing age of the leaves. In the case of diseased leaves, however, the percentage of calcium in the water-soluble fraction of the dry matter shows increases of much greater magnitude. In the dry matter of mature healthy walnut leaves practically all of the calcium is water-insoluble. This is not due to precipitation of the calcium because of any development of alkalinity in drying

the leaves, as a water extract of the ground twice-dried leaves of healthy trees gave a pH value of 4.84, which is somewhat more acid than the juice of healthy leaves. The percentage of magnesium in the ash of the water-soluble fraction of the dry matter of the walnut leaves increases with increasing age of the leaves, while that of potassium shows a decrease.

Table VI indicates that diseased walnut leaves may have a lower percentage of calcium in their ash than healthy leaves, but that the percentage of water-soluble calcium may be greater in the diseased than in healthy leaves, and it suggests that some toxic agent in the diseased leaves prevents some of the water-soluble calcium from becoming water-insoluble.

It was of interest to express the juice of frozen mature healthy and diseased leaves of walnut and make a comparison with that of healthy orange leaves. Such a comparison is presented in table VII, which shows that the ash content of expressed juice of orange leaves is from three to four times as concentrated as that of walnut leaves. The percentage of sodium, potassium, and magnesium in the ash of walnut leaf juice is much greater than that of orange leaf juice. The striking comparison is that the percentage of calcium in the ash of the expressed juice of orange leaves is about fifteen times as great as that of walnut leaf juice.

If we calculate the total amounts of the ash constituents from the data given in table VII, we find that the total amount of potassium in the expressed juice of walnut as compared with that of orange leaves may not differ greatly; however, the total amount of calcium in the expressed juice of walnut as compared with orange leaves is extremely low. KELLEY and CUMMINS (2) found a marked decrease in the percentage of calcium in the ash of expressed juice of citrus leaves when mottled as compared with that of normal leaves. We have found only a small difference, however, between the composition of the ash of the expressed juice of healthy and diseased walnut leaves.

KELLEY and CUMMINS also found the expressed juice of mature normal citrus leaves to have a greater pH value and less total acidity than the expressed juice of mottled leaves. In these respects the results for the expressed juice of normal and mottled citrus leaves





were analogous with those we have obtained for the juice of healthy and diseased walnut leaves, the data for which are set forth in table VIII.

TABLE VII

COMPOSITION OF EXPRESSED JUICE OF LEAVES FROM HEALTHY AND DISEASED WALNUT TREES COMPARED WITH JUICE OF NORMAL MATURE ORANGE LEAVES

	WALNUT LEAVES		ORANGE LEAVES
	Healthy	Diseased	Healthy
Ash (gm. per 100 cc. of juice)			
	1.3516	1.5948	5.3325
Ash constituents (percentage of ash)			
Na.....	9.20	9.66	4.18
K.....	44.18	43.07	14.49
Ca.....	1.85	1.57	24.98
Mg.....	5.67	5.26	2.39
Cl.....	11.24	13.06	.....
SO <sub>4</sub> .....	2.92	3.36	5.15
PO <sub>4</sub> .....	6.88	10.49	2.09

TABLE VIII

RELATION OF pH AND TOTAL ACIDITY OF 5 CC. SAMPLES OF EXPRESSED JUICE OF LEAVES FROM HEALTHY AND DISEASED WALNUT TREES

TOTAL ACIDITY (cc. of N/10 NaOH)	pH	
	Healthy	Diseased
0.....	5.14	4.36
1.15.....	6.91	5.65
1.73.....	7.13	.....
2.30.....	7.46	6.49
2.88.....	7.68	.....
3.46.....	.....	7.29
4.04.....	8.13	7.51
5.19.....	.....	7.91
6.34.....	.....	8.18

Samples of sap from the trunks of healthy walnut trees were collected by boring a slightly inclined hole into the trunk and attaching a threaded block-tin pipe, attached to a Pyrex flask protected from dirt, light, evaporation, etc. The pH of some samples

was as high as 8.2, and changed to 5.2 on standing. The pH of sap forced by means of compressed air out of the ends of freshly cut walnut shoots showed a pH of 5.2. The buffer value of the sap is probably very small, and fluctuations in the  $\text{CO}_2$  content of the sap possibly account for the great variations.

Table IX shows the analysis of the 365 cc. sample of sap obtained by natural flow. The  $\text{PO}_4$  determination was made on a separate 34 cc. sample of sap from the same tree. The potassium, calcium, and phosphate content are considerable.

To supplement the data of table IX we have obtained branches from healthy and diseased trees, and roots from healthy walnut

TABLE IX  
COMPOSITION OF SAP OF NATURAL FLOW OBTAINED BY  
TAPPING A HEALTHY WALNUT TREE

ASH FROM 365 CC. SAP	0.1686 GM. OR 462 PARTS PER MILLION	
	Ash constituents (percentage ash)	Parts per million in sap
Na.....	5.88	27
K.....	22.83	105
Ca.....	18.80	87
Mg.....	4.15	19
Cl.....	0.84	4
$\text{SO}_4$ .....	1.46	7
$\text{PO}_4$ .....	21.15	98
Total S as $\text{SO}_4$ .....		38

trees (*Juglans hindsii*). Adapting the fundamentals of the method in use in Dr. BENNETT's laboratory at Berkeley, we have withdrawn the sap from the branches and roots by means of a battery of six Pyrex suction flasks and the use of air suction.

Table X gives the results of the analyses of the sap obtained by suction. It is seen that a very close agreement exists in the concentration of the potassium from branches of healthy and diseased trees. The outstanding fact is the large concentration of calcium in the sap of the diseased as compared with that of the healthy branches. This agrees with the results on water solubility of the calcium of the dry matter of diseased walnut leaves as shown in table VI. Possibly the larger concentration of calcium in the

branches of the diseased trees is due to its accumulation as a result of the inability of the diseased leaves to utilize it in as great amount as normal leaves.

TABLE X

COMPOSITION OF SAP OBTAINED BY SUCTION FROM HEALTHY AND DISEASED  
WALNUT TREES (PARTS PER MILLION)

	HEALTHY BRANCHES	HEALTHY BRANCHES	DISEASED BRANCHES	HEALTHY ROOTS	HEALTHY ROOTS
Total solids .....	8105	4040	5176	4134	2328
Ash.....	653	565	885	.....	.....
Na.....	48	.....	127	5	.....
K.....	113	.....	111	109	.....
Ca.....	131	126	258	87	.....
Mg.....	.....	73	31	.....	.....
Total P as PO <sub>4</sub> .....	.....	.....	.....	136	16

### Discussion

We have shown that yellows or little-leaf of walnut trees has much in common with mottle-leaf of citrus, rosette of pecan, etc. For a practical demonstration of this fact we have selected the area of soil where plots G and H adjoin on the Rubidoux tract. Plot G has been fertilized annually for the past 13 years at the rate of 486 pounds of nitrate of soda, 500 pounds of dried blood, and 1067 pounds of steamed bone meal per acre per year. During the 7-year period prior to this, the same fertilizer materials were applied but in smaller amounts. Contiguous to plot G is plot H, which has been fertilized annually for the same period at the rate of 972 pounds of nitrate of soda per acre per year. The citrus trees which are growing on the land where these two plots adjoin each other are at present badly affected with mottle-leaf. In the interspace where these two plots adjoin we planted in the spring of 1925 several normal trees each of walnut, pecan, peach, and apricot.

The walnut trees are now badly affected with yellows or little-leaf. The apical growth is badly rosetted, and the leaves are either green with mottling very conspicuous, or are pale yellowish green and folded. The pecan trees show extreme cases of rosetted leaves, and the peach trees show typical little-leaf, which can be distinguished at a considerable distance. The peach leaves are narrow

and long, and frequently are mottled and folded dorsally along the midrib. The apricot trees are typical of the condition of many trees that are found growing in commercial orchards. Many if not all of the leaves of a cycle may be considerably reduced in size, while those of an adjoining cycle may be much larger. It is not uncommon to find considerable mottling among the smaller leaves.

Regardless of what may be designated the cause of mottle-leaf of citrus, we have shown herein that various troubles of the other kinds of trees used in these tests may be brought about by the same environmental conditions. On the other hand, when trees of the same lot were planted in soil in which citrus is free from mottle-leaf, no troubles of the kind described occurred. This strongly indicates that these troubles are due to soil conditions and not to infection. Controlled cultures in sand and soil are being conducted by the senior author, so as to learn more specifically the nature of these "physiological" tree diseases.

As to what has taken place in the soil of plots G and H owing to the fertilization, one can only speculate as yet. The bases of the soil no doubt have been undergoing exchange with the bases (principally sodium) in the fertilizer applied, with the result that calcium, magnesium, and potassium have been set free into the soil solution and sodium has largely taken their places in the soil complex. The bases of the soil solution may be absorbed by the roots, or carried below the reach of the tree roots by excessive irrigation or by rains, or may partially remain bathing the roots. In this way the roots are gradually being given a very different environment from that originally present. In soil cultures, by a replacement of the bases in the soil by means of potassium nitrate solution, it has been possible to produce rosetted pecan leaves.

The sodium complex may hydrolyze, moreover, and alkalinity may develop. The solubility of the iron, calcium, phosphate, etc., may thereby be gradually reduced. In line with this idea, the senior author is now conducting experiments with soil in which alkalinity is being developed by mixing sodium carbonate, potassium carbonate, or sodium aluminate with the soil and then planting budded citrus trees. Orange trees have been caused to mottle badly while the control trees are healthy. In this case no removal of bases by

leaching has occurred, and the only conclusion to be drawn is that alkalinity has prevented the trees from a full utilization, possibly by effects within the tree itself, of the bases (largely calcium) most needed in the maturing of the leaves.

One other possibility suggests itself, and that is that there may possibly be sufficient absorption of toxic agents, in exceedingly small amounts, to cause these leaf diseases. This possibility cannot be dismissed lightly, for experiments under way suggest that leaves affected with such toxic agents may be prevented from obtaining sufficient calcium to enable them to mature, and consequently may develop a condition resembling these physiological diseases.

The writers are convinced that in the soil of plots G and H, and in other experiments where the diseases considered are prevalent, there is abundant nitrogen, and that therefore nitrogen starvation cannot be a factor.

### Summary

1. The yellows or little-leaf of walnut trees and the rosette of pecan trees, as well as little-leaf of the peach and mottling of apricot and of citrus, have been found to be independent of the nitrogen requirements, the anion content of the water extract of the soil, climatic factors, cultural and irrigation practices, nematode infestations and soil types, but appears to be dependent upon the base relationship in the soil. These tree diseases are "physiological" and not due to infection.

2. The ash of affected leaves is lower in calcium and higher in potassium than that of healthy leaves of the same age.

3. The percentage of calcium in the ash of the water-soluble fraction of diseased leaves increases as maturity is approached, and finally becomes considerably greater than that of healthy leaves. Nearly all of the calcium of the dry matter of healthy walnut leaves is insoluble in water.

4. The sap drawn by suction from diseased walnut branches shows a greater concentration of calcium than that from healthy branches.

5. The ash content of walnut leaves increases very rapidly at the outset of growth, and then shows a slow but progressive increase until maturity is reached, after which there may be a slight decline.

By July 1 the ash content of the dry matter is about three-fourths that at maturity.

6. The expressed juice of orange leaves is from three to four times as concentrated as that of walnut leaves in ash content. The percentage of calcium in the ash of the expressed juice of orange leaves is about fifteen times as great as that of walnut leaves.

7. Practically no differences exist in the ash composition of the expressed juice of healthy and diseased walnut leaves.

8. The expressed juice of diseased walnut leaves has a lower pH value and a greater total acidity than that of healthy walnut leaves.

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# CHEMISTRY OF GROWTH AS REPRESENTED BY CARBON/NITROGEN RATIO<sup>1</sup>

## REGENERATION OF WILLOW CUTTINGS

PHYLLIS A. HICKS

(WITH EIGHT FIGURES)

### Introduction

The power of regeneration is to some extent common to all living organisms, but the factors governing regenerative response are still insufficiently understood, and a general theory of regeneration is hardly possible at the present state of knowledge. Even regarding the simplest form of regeneration, namely, the production of a shoot from a dormant bud, there are conflicting views.

PFEFFER (20) believes that there are two forms of rest period, autonomic, or internally caused, and aitionomic, or externally caused. KLEBS (11), however, maintains that the cause is entirely external, while HOWARD (6) states: "The beginning of rest, particularly in woody plants, appears to be wholly due to inner causes, but before the complete state of rest is reached outer conditions may help to hasten the rest." In support of his statement, KLEBS brought hyacinth and celandine into a hotbed in the greenhouse, checked the resting period, and had two flowerings a year. KLEBS is supported by FLAMMARION (3), who grew seedlings from acorns under greenhouse conditions for fifteen years, by the end of which time the young trees became almost like evergreens, the new leaves appearing before the fall of the old ones.

If the rest period were caused entirely by external conditions becoming unfavorable to growth, it should be broken upon transfer of the plants to favorable conditions. HOWARD (6), however, has proved that many plants fail to grow when brought into a warm greenhouse, either in November or January. MOLISCH (16) used warm baths and radium, JOHANNSEN (8) and STUART (26) ether or chloroform, JESENKO (9) weak alcohol, MCCALLUM (18) ethyl bro-

<sup>1</sup> Part of thesis accepted for the degree of Ph.D. of the University of Wales.

mide, HARSHBERGER (5) ammonia, BOS (1) a galvanic current, LAKON (12) various nutrient solutions, and WEBER (28) mechanical injury. Even these many and varied expedients failed to stimulate growth in all cases, although they were effective in many at certain times of the year. Thus the theory of external causes is not adequate.

Internal conditions, such as sugar or starch accumulation, have been held responsible by the supporters of the autonomic hypothesis, but analyses have shown that the rest period begins or is broken quite independently of the amount of these substances present; and, indeed, the amount of these compounds is different every year. These facts lead us to suppose that neither external nor internal conditions alone are responsible for initiating or breaking the rest period, but that they interact.

As regards the physiology of the plant, the rest period is not a phase of entire inaction. As a result of his experiments, SIMON (24) states: "So far as some of the functions of growth and metabolism and also respiration are concerned, woody plants have no rest period, for even in winter under favorable conditions these activities reach a relatively high intensity." Similarly, FISHER (4) shows that the reserve materials in most trees undergo marked transformation during that period. Thus with the study of regeneration, in so far as it concerns the opening of buds only, we have not to deal with "awakened" metabolism, but merely with stimulated processes.

SMITH (25) suggests the possible importance of the C/N balance in regeneration, while SUMMERS (27) discusses the possibilities of this more fully, and, quoting the memoir of BUTLER, SMITH, and CURRY (2), concludes that the awakening of vegetation is synchronous with the translocation of nitrogenous reserves to the growing regions.

MÜLLER-THURGAU (17), working on potatoes, has shown that growth is preceded by sugar being made available as fuel for the greatly increased respiration, the sugar being formed by diastatic ferment of reserve starch or other carbohydrate. This is substantiated by FISHER's statement that resting buds of trees cannot be made to grow until a certain minimum of insoluble starch has been changed "to easily respired and therefore energy yielding material."



JOHANNSEN and PFEFFER entirely disagree that the presence of sugar is a controlling factor, while other workers show that increased respiration is not alone responsible for regeneration. This again points to the value of a "food" balance as a possible controlling agent.

In considering regeneration of roots as well as shoots, other important factors demand consideration. The first of these is polarity. One of the greatest workers on this problem has been, without doubt, LOEB (13). As a result of a great number of experiments on *Bryophyllum*, he accounts for the tendency of all cuttings to form shoots at the apex and roots at the base in the following way. Specific hormones or formative materials are supposed to exist in the plant, each responsible for the growth of a particular type of structure. Thus the growth of roots at the lower end of the shoot is assumed to be due to flow of root-forming substances to the lower end of the cutting, and shoot-forming substances to the apex. Inversion of the shoot, however, will cause a reversed polarity after a time, so that the supposition that the shoot-forming substances were specifically lighter than those responsible for root production is inadequate. The objection that, on severing a long stem into several cuttings, the apex of one is practically the base of the next, was surmounted by assuming that the redistribution of the hormones may be modified by gravity, as, for example, through drainage of root-forming materials away from the anterior cut end of the posterior piece, rendering the growth of roots impossible, while the root-forming materials are unable to escape from the posterior cut end of the anterior portion. The presence of leaves upon the cutting affects root formation and geotropic curvature in amount, according to their basal or apical situation on the stem. How far this hypothesis of LOEB may be modified by the investigation of changes in the C/N ratio will be discussed later.

### Investigation

This investigation upon the variation of the carbon and nitrogen relations during normal regeneration, together with the effects of increasing and decreasing the initial C/N ratio, was begun in February 1927.

Cuttings from the winter shoots of a variety of *Salix viminalis* from the Roath Park Botanical Gardens were suspended in Kilner jars containing a little distilled water, lined with blotting paper also moistened with distilled water. Two sets of normal cuttings were arranged, one set suspended in moist air only, and the other with the base of the cutting dipping into the water.

Previous workers had found that regeneration was stimulated by etiolation, a fact which they thought to be due to the reduction of carbon, and hence of the C/N ratio. Analysis, however, showed that the etiolated plants were full of starch.

Another attempt at discovering the effect of sugars upon regeneration had been made by placing a leafy shoot into sugar solution, transpiration thus supplying the pull. Unfortunately the sugar passed into the leaves, which then dropped off. In the present experiment sugars were drawn into the shoot by means of a suction pump, and the cut ends sealed with a rubber tube and glass cap. Prior to this the amount of carbon and nitrogen already in the stem had to be ascertained. The epidermal layer was removed and the underlying tissues, consisting of cortex, phloem, and cambium, were scraped off, dried to constant weight at 100°C., and the two elements estimated by the PRÉGL (21) micro-analytical methods described fully in part I.<sup>2</sup> Total elemental nitrogen and total elemental carbon were thus estimated. These tissues, afterward spoken of collectively as "bast" tissues, were chosen as representing the chief seat of regenerative activity. SMITH has shown that the adventitious roots in *Coleus* arise from a nest of highly meristematic cells in the cambium, the appearance of which seems to be followed by enzyme action, since sections show a gap suggestive of digestion in the area of the advancing root tip. KLEBS also uses these tissues to determine enzyme activity during regeneration, the wood being discarded as showing too slow an enzyme action. A very few estimations of the wood, however, were made in this study.

The bast tissues were found to be low in nitrogen, 1.623 per cent, and high in carbon, 34.48 per cent (C/N 21.2). This high carbon value is due chiefly to salicin, which is stored in the cortex of the

<sup>2</sup> HICKS, PHYLLIS A., The carbon/nitrogen ratio in the wheat plant. *New Phytol.* 27: April 1928.

willow. The salicin is normally split nightly into glucose and saligenin, but in autumn it tends to accumulate, owing to the reduced activity of the enzyme salicase. Thus glucose is the translocation product of salicin, a fact which will be of importance later.

The second series of cultures was then so arranged that the shoots possessed at least double the C/N ratio existing in the bast tissues of the normal shoots. The possible difference of availability of several sugars was allowed for, and glucose, saccharose, lactose, and levulose, together with the glucoside salicin, were used. The strength of the solution which had to be drawn up by the twig to produce the desired increase of carbon content was calculated as shown by the following example.

1. RELATION OF DRY WEIGHT OF BAST TO WET WEIGHT OF TWIG:

Wet weight of twig..... = 0.7263 gm.

Wet weight of bast in twig..... = 0.1283 gm.

Dry weight of bast in twig..... = 0.07 gm.

Ratio:  $\frac{\text{Dry weight bast}}{\text{Wet weight twig}} = \frac{0.07}{0.72} = \frac{1}{10}$  (approximately).

2. AMOUNT OF LIQUID DRAWN UP BY AVERAGE TWIG:

Experiment performed with water. Twig measuring 5.7 inches and having a wet weight 2.626 gm. took up 1.5 cc. of water.

3. ASSUME: (a) In all twigs there is a similar proportion of dry weight of bast to wet weight of twig ( $\frac{1}{10}$ ); (b) all twigs will draw up a proportionally similar amount of liquid; (c) all tissues of the stem will receive an equal amount of solution.

4. CARBOHYDRATES USED:

Glucose.....	$C_6H_{12}O_6$ .....	Unit molecular weight 180
Levulose.....	$C_6H_{12}O_6$ .....	Unit molecular weight 180
Saccharose....	$C_{12}H_{22}O_{11}$ .....	Unit molecular weight 342
Lactose.....	$C_{12}H_{22}O_{11}$ .....	Unit molecular weight 342
Salicin.....	$C_{13}H_{18}O_7$ .....	Unit molecular weight 286

Let 100 gm. of bast tissue be increased by 50 gm. of carbon: then 0.2626 gm. ( $\frac{1}{10}$  wet weight twig) will be increased by 0.1313 gm. carbon; twig takes up 1.5 cc. of solution; therefore, 1.5 cc. of solution must contain 0.13 gm. carbon.

72 gm. carbon are contained in 180 gm. glucose and levulose.

72 gm. carbon are contained in 171 gm. lactose and saccharose.

72 gm. carbon are contained in 132 gm. salicin.

0.13 gm. carbon are contained in  $\frac{0.13}{4}$  gm. glucose and levulose

0.13 gm. carbon are contained in  $\frac{0.541}{24}$  gm. lactose and saccharose

0.13 gm. carbon are contained in  $\frac{0.143}{6}$  gm. salicin; therefore 100 cc. of solutions must contain 21.6 gm. glucose and levulose, 20.6 gm. saccharose and lactose, and 15.9 gm. salicin.

In the case of saccharose and glucose some twigs were also suspended so that they dipped into a solution of the preceding strength, in lieu of having it injected. The provision of nitrogen for decreasing the ratio was similarly calculated, a 0.2 molecular solution of potassium nitrate increasing the nitrogen content by 1.6 per cent. In the case of this series, also, a set of uninjected cultures dipping into the same solution was arranged. Cuttings with decreased and increased ratio were set up in Kilner jars prepared as for normal cuttings, and all placed under similar conditions of illumination and temperature in the greenhouse.

### Morphological results

Up to the tenth day, when the first samples of bast tissues from the base of the stem were taken, no morphological change was observed, and it was not until after fourteen days that root initials were formed on one of the cuttings dipping into water, and the shoots of the carbon-infused cultures began to open. From that day bud development was rapid in the sugar cultures. On the fifteenth day the order of development was as follows:

Lactose and glucose (almost alike)

Salicin

Saccharose and levulose (no growth).

The next day the buds of the saccharose-infused cuttings showed a slight tendency to bursting, and on the seventeenth day the order was:

Glucose, well developed shoots with root initials established  
Lactose, also with root initials  
Salicin, no roots  
Saccharose, slight growth, no roots  
Levulose, no development.

By this time the buds were beginning to unfold in the normal cultures, and roots were also forming. The nitrate cultures showed no sign of development, but exhibited a marked tendency to develop molds. By the twenty-eighth day the difference between the three series of cultures was very striking. The nitrate cuttings were still undeveloped, the normal cultures were well developed, the cuttings suspended in water being further advanced in shoot and less advanced in root development than those suspended in moist air. In the former all roots developed above the water line.

The sugar series was about twice as advanced as the normal cultures, both in root and shoot development. Long shoots with well expanded leaves were shown by the glucose, saccharose, and levulose cultures, which latter, although last in bursting, grew very quickly and surpassed the lactose and salicin cultures. The cuttings hanging into glucose and saccharose showed also well developed shoots but as yet no roots. Six days later roots developed, but at the top of the cutting and above the shoots. This reversed polarity was shown by the cuttings suspended into both glucose and saccharose. Whether this is an accidental peculiarity of these individual cuttings, or whether the difference may be constant, is at present under investigation.

Many of the sugar-infused cultures showed roots all over the stem, that is, poorly marked polarity. The nitrate-infused cultures were badly molded, but a few buds were beginning to develop, and in one case a root had emerged (as in the sugar cultures) at the top instead of the base. This root, the only one developed by the nitrate cultures, grew to about 0.25 inch and then died off. The shoots were extremely backward, and after bursting the bud developed no further (figs. 1, 2).

By the end of the next fortnight, etiolation was strongly marked in all the sugar cultures, which had reached their maximum develop-

ment; less so in the normal cultures; while the normal cuttings dipping into water were fresh and green. This fact is probably related to the greater water supply and the consequent easier translocation of the products of metabolism. Even at this date the nitrate cul-



FIGS. 1-3.—Winter shoots of *Salix viminalis*: fig. 1, injected with  $\text{KNO}_3$  (suspended in moist air); fig. 2, normal (suspended in moist air); fig. 3, injected with glucose (suspended in moist air).

tures were still undeveloped, showing very few root initials and hardly any bud growth.

These results are apparently in line with those of Miss REID (22). She grew carbon-high and nitrogen-high cuttings of tomato in light and darkness, and in solutions with and without nitrates, and in every case the carbon-high plants grew rapidly, while the nitrogen-high cuttings showed very poor root and shoot development or none at all.

These morphological observations seem unusual in view of the

facts that nitrogenous manures applied to plants stimulate rapid growth; and that, judging from the results of the experiments on wheat, embryonic and young growing parts are rich in nitrogen; or the explanation of MURNEEK (19) of the inhibition of vegetative growth by fruit setting in the tomato as due to the monopoly of the soluble nitrogen by the fruit and the consequent accumulation of carbohydrate in the vegetative parts. It stresses the fact, however, that it is the ratio of carbon to nitrogen which is of prime importance, and not the amount of either element present.

The explanation of some of these results will be considered later.

### Statistical results

Tables I-III show the main trend of the C/N relation during regeneration, and the contrast between the three sets of cultures. Apparently the first stimulus to regenerative germination, combined with the external conditions of moisture and warmth, is the presence of free glucose or lactose, both of which are very easily respirable sugars. Salicin itself is not directly respired, but is turned into glucose before utilization by the willow. Thus cuttings infused with glucose and lactose respired rapidly. Respiration provides the necessary energy for the withdrawing of reserve nitrogenous material from the wood and pith, and their upward translocation. Thus the beginning of growth is marked by an increase in the nitrogen percentage of the phloem tissues. This is most marked under the buds. The C/N ratio falls considerably, due to decreased carbon and increased nitrogen.

The nitrogen drawn into the bast tissues is then translocated upward, so that the tip becomes richer in this element than the base of the shoot. On the other hand, the carbon is withdrawn, more particularly from the upper region, by the increased respiration of the unfolding buds.

Whether a part of the large amount of sugar drawn into the cutting passed into the tissues of phloem, cambium, and cortex is not seen from these analyses, begun ten days after setting up. However, judging from comparisons with the nitrate cultures, and from the results of the more slowly developing saccharose culture, there is a probability that this is the case. In the case of saccharose, after

TABLE I  
NORMAL CUTTINGS

	MOIST AIR			WATER		
	Carbon %	Nitrogen %	C/N	Carbon %	Nitrogen %	C/N
Undeveloped twig						
Bast tissues.....	34.479	1.623	21.2	34.479	1.623	21.2
Wood.....	40.49	1.963	21.7			
Bud.....	31.778	5.735	5.5	31.778	5.735	5.5
10 Days						
Bast tissues.....	34.18	1.819	18.9	34.23	3.621	9.5
Root initial area, bast.....	34.55	2.089	17.2			
19 Days						
Root area, bast tissues.....	34.00	1.801	18.8			
Young root.....	28.701	5.941	4.8			
Unfolding shoot.....	30.714	6.391	4.8			
Bast tissues around shoot.....	37.63	3.002	12.5			
28 Days						
Base of shoot, bast tissues.....	33.756	2.917	11.6			
Older root.....	29.106	5.944	4.9			
Older shoot.....	34.624	5.310	6.6			
Bast tissues around shoot.....	34.00	2.914	11.7			
37 Days						
Base of shoot, bast tissues.....	30.221	3.504	8.1			
Tip of shoot, bast tissues.....	27.841	3.793	7.3			
Under bud, bast tissues.....	33.615	2.614	12.9			
Wood.....	36.138	1.514	23.9			

TABLE II  
NITRATE CULTURES

	ONE-FIFTH MOLECULAR POTASSIUM NITRATE INJECTED CULTURES			HANGING INTO ONE-FIFTH MOLECULAR POTASSIUM NITRATE		
	Carbon %	Nitrogen %	C/N	Carbon %	Nitrogen %	C/N
Undeveloped						
Bast tissues.....	34.479	1.623 + possible 1.6	?	34.479	1.623	21.2
10 Days						
Bast tissues.....	31.3	2.651	11.8	34.045	(?) 3.794	(?) 7.9
19 Days						
Bast tissues.....	30.764	2.372	12.9			
28 Days						
Bast tissues.....	34.215	3.998	8.5	26.136	1.952	13.5
37 Days						
Bast tissues, base of stem.....	31.256	2.523	12.4			
Bast tissues, tip of stem.....	30.744	3.699	8.3			
Bast tissues, under bud.....	32.045	3.023	10.6			



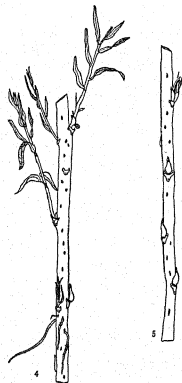


ten days the carbon percentage of the bast tissues had risen from 34 to 37 per cent. This is interesting since in fruit trees (for example, apple) saccharose is looked upon as a special form of storage, with special functions at bud-break. The saccharose and levulose cultures at first were slower in developing, presumably due to greater difficulty in the respiration of these sugars. However, once the C/N

ratio had been lowered to within the growing limits (ratio probably below 15), growth of these two cultures was vigorous and rapid.

In the normal cultures respiration was much slower, and in ten days only 1 per cent of the carbon had been respired. This meant that the nitrogen translocation was also slower, and it thus took longer for the "growth ratio" to be established. The cuttings hanging in water contained after ten days much more nitrogen than the other normal cuttings, an abundant supply of water evidently being an important factor in the translocation of this element into the phloem. The low C/N ratio so produced favors shoot formation rather than root development.

In the case of the nitrate cultures the injected nitrate passed into the bast tissues, but the carbon content of the bast tissues was not lowered by respiration. The cut-



FIGS. 4, 5.—Winter shoots of *Salix viminalis*: fig. 4, suspended in water; fig. 5, suspended in one-fifth molecular  $\text{KNO}_3$ .

ting had evidently been thrown into Class I of KRAUS and KRAYBILL (10), the nitrates being excessive; and, in contrast with the case of respirable carbon compounds, the plant has no means of removing them, so growth was checked.

As in the case of flowering, so in the regenerative germination it is the ratio of the C/N which is all important, and not the relative amount of either element.

One question which cannot be avoided is where the nitrogen in the new growths comes from. Chemical analysis shows that in part

it is withdrawn from the wood and pith, but since the percentage is almost doubled, even after rapid growth has taken place, it is doubtful if the amount in the wood and pith is sufficient to cause such an increase. Can the nitrogen of the atmosphere play some part in green plant metabolism after all?

The young roots are rich in nitrogen and somewhat low in carbon, and as they develop, the normal increase in the C/N ratio is followed. The undeveloped buds contain a very high percentage of nitrogen, and the lowest C/N ratio of the whole cutting. As they develop, the nitrogenous material is piled up in the tissues around the base of the growing shoot, so that a low C/N ratio is maintained by the shoot for a considerable time.

#### New polarity hypothesis

In the mature long stem, while attached to the branch of the tree, there is relatively little gradation in either carbon or nitrogen, although the percentage of the latter element is slightly higher in the apical portion. On severing the stem into portions and hanging each portion in moist air in a warm room, activated respiration seems to supply energy to expedite the translocation of nitrogenous compounds. The nitrogen is translocated upward, the carbon flow being downward. Thus in any cutting prior to growth a lower C/N ratio is established at the apex, with a higher ratio at the base and a gradation in between. Shoots



FIGS. 6, 7.—Winter shoots of *Salix viminalis*: fig. 6, suspended in saccharose; fig. 7, suspended in glucose.

are formed in the region of the lowest C/N ratio; roots can and do form where the higher ratio occurs.

This gradation in C/N ratio can be translated into terms of LOEB's "root forming and shoot inhibiting" hormone (a high C/N ratio), and "shoot forming and root inhibiting" hormone (a low C/N ratio). The absolute values of the elements are of little importance

compared with the ratio, so that severing a stem leaves the gradient of the ratio in each piece unaffected.

When a cutting is inverted, a lag is necessitated, possibly owing to the reversed position of the vessels of the wood and bast, but the vessels maintain their normal action, the nitrogen flow now being downward, the carbon upward. Thus the morphological base has the highest C/N ratio and bears roots; the true apex with a lower C/N ratio produces shoots.

If the shoot is maintained in the horizontal position normal polarity is maintained, since translocation of nitrogen is not interfered with. The influence of the leaf upon root formation as reported by LOEB (13) can also be explained on this basis. LOEB states that if on a horizontally suspended stem of *Bryophyllum* (in which the growing point is cut off) one leaf is pre-



FIG. 8.—Shoots: *a*, normal (suspended in water); *b*, sugar (suspended in glucose).

served at the apex, strongly marked geotropic curvature occurs with extensive root formation at every node of the stem.

If the single leaf is basal the curvature is considerably less, however, and the little root formation which occurs is confined to the nodes of the basal leaf and the internode below. These effects can be altered by incisions, or by removing the cortex on one side of the stem. This is explained in the following way.

The respiratory activity of the leaf appears to create a suction

upon all the tissues below it. Thus the ratio of carbon to nitrogen in these tissues is reduced by carbon loss. The pull of transpiration, too, causes the nitrogen to rise in the stem. Thus the C/N ratio gradient is maintained, while the ratio itself below the leaf is lowered, so that growth of roots is established. Again, since a lowering of the C/N ratio means changing the plant into a chemically younger state; geotropic curvature is thus increased, younger tissues being more responsive than mature ones.

If the leaf is at the base, however, only the portion below it will experience the suction, due to respiration and transpiration, and only this part will be turned into a regenerating region. The tissues of the stem above the leaf are thus chemically old, and hence less responsive to geotropism. Incisions naturally affect the response since they restrict the upward flow of nitrogen.

This hypothesis can also be used to explain the production of roots at the apex of the cuttings suspended in sugar. In this case the shoots developed first, and transpiring actively drew up the sugar solution which necessarily accumulated at the top of the cutting. Thus the C/N ratio became highest at the top, and roots consequently developed in that area. This assumption, unlike the primary hypothesis, is not supported by chemical statistics, but is at present under investigation.

These results are not in any way complete: more abundant data are required at more frequent time intervals, particularly at the beginning of the experiment, but they are submitted as a preliminary outline of the relation of the C/N ratio to regeneration.

### Summary and conclusions

1. Experiments on the regeneration of cuttings of *Salix viminalis* have been carried out with normal cuttings, and with cuttings injected with potassium nitrate and various sugars.
2. The initiation of growth is due to stimulated respiration, giving energy for the withdrawal of nitrogen into the bast, and its consequent upward translocation, particularly to the buds. The injected sugars hasten this considerably, especially easily respirable

sugars such as glucose and lactose. Potassium nitrate in excess prevents the commencement of growth.

3. LOEB's hormone hypothesis is explained as a gradation of the C/N ratio throughout the cutting. Shoots grow at the area of the lowest C/N ratio, roots at the higher. The effect of a leaf at the apex or base of the shoot is also interpreted on this basis.

This work was carried out in the botanical department of the University College of South Wales, Cardiff, under the direction of Professor McLEAN, to whom I wish to express thanks for his interest and suggestions during its procedure.

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# EFFECTS OF ULTRA-VIOLET RADIATION ON VARIOUS FUNGI

F. L. STEVENS

(WITH TWELVE FIGURES)

## Introduction and general methods

As announced in a preliminary note,<sup>\*</sup> ultra-violet radiation has a striking and profound effect upon the reproductive processes in various fungi, calling forth immediate and profuse sexual or conidial development in fungi that normally exhibit these stages only very rarely. The present article gives in some detail the studies upon which the preliminary report was based, as well as those of subsequent experiments.

The method of procedure unless otherwise stated was as follows: Full radiation from a Cooper-Hewitt quartz mercury arc operated at 4.5 amp. and 66 volts was employed. The agar plate cultures with the Petri dish covers removed were placed directly in front of the source of light, at a distance of 21 cm. A shield of hard rubber from a photographic plate holder was placed in front of the plate, in order to shade certain portions of the colony, usually one-half, from the light.

Difco corn meal agar was employed, 10-12 cc. per Petri dish. Inoculations in early experiments were made by transferring a bit of mycelium-bearing agar to the center of a solidified plate. Later it was found to be more expeditious and satisfactory to make a suspension of conidia in a drop of sterile water and transfer an oese of this suspension to the agar plate. Cultures used in the experiments were 4-7 days old.

I wish to acknowledge my great indebtedness to the Department of Physics of the University of Illinois for the use of apparatus, and to Professor JACOB KUNZ and F. W. COOKE for suggestions and helpful assistance.

<sup>\*</sup> The sexual stage of fungi induced by ultra-violet rays. *Science* N.S. 1923.



*Glomerella cingulata*, strain G 10

This strain, *Glomerella cingulata* (Stoneman) Spaulding & von Schrenk, strain G 10, originally isolated from apples affected with bitter rot, was most extensively used in these studies, and the following account is based upon experiments with it. A single conidium was isolated in October, therefore G 10 as treated in these accounts is of monosporous origin. Normally as cultured on agar it develops luxuriantly at the rate of about 4 mm. per day radially, thus completely occupying a Petri dish in about 13 days. G 10 in one large series of cultures kept under observation until 30 days old was not seen to produce perithecia in the monosporous strain on agar or apples. In very old cultures the original strain did occasionally bear a few sclerotoid masses 2-3 mm. in diameter, which produced numerous perithecia. Such cultures closely resembled those figured by SPAULDING and VON SCHRENK, except that the perithecial sclerotia were much less numerous. The cultures also differed from their descriptions in that the perithecia were not imbedded in a stroma, but rather consisted of numerous, nearly globose perithecia of neat contour, closely grouped, but which were readily separated by crushing the mass.

Conidia, when placed upon agar, showed but the slightest indication of germination in 6 hours. In 22 hours, however, they had developed filaments 0.7 mm. long along the surface of the agar, and 0.8 mm. long penetrating into the agar. Thus in agar 1 mm. deep 24 hours suffices for the mycelium to penetrate to the glass below.

## EFFECTS OF ULTRA-VIOLET RADIATION

When colonies 2-7 or 8 days old are radiated with the appropriate dosage, death and collapse of the aerial mycelium is immediate, growth of the colony is temporarily stunted, and perithecial formation is induced both by the direct and by diffused rays.

LETHAL EFFECTS.—To determine the lethal dosage upon spores, two series of 14 plates each were arranged: one with the spores placed upon the top of the agar, the other with the spores covered by the agar, the agar layer being approximately 1.5 mm. thick. Each series was radiated with exposures varying from 5 to 90 seconds. With a treatment of 5 seconds only a few of the unprotected

spores germinated and these but poorly. With an exposure of 10 seconds still less germination was noted, although in both instances vigorous colonies resulted. With an exposure of 15 seconds or more no germination resulted. The spores protected by the agar layer, subjected to an exposure of 5, 10, and 15 seconds, gave good germination; 20, 25, and 30 seconds gave fair germination; 35, 40, 45, and 50 seconds gave poor germination; 60, 70, and 80 seconds gave very poor germination; 90 seconds gave still fewer germinating spores, but still sufficient to result in a vigorous colony.

The minimum lethal dosage for uncovered spores, therefore, lies somewhat over 10 seconds but less than 15 seconds. The lethal dosage under 1.5 mm. of agar lies at slightly more than 90 seconds.

The lethal effect on the aerial mycelium is at once evident, especially in regions bearing considerable floccose mycelium, in that all such mycelium immediately falls flat upon the agar, thus giving the radiated half of the colony a strikingly different appearance from that of the non-radiated portion.

**COLONY STUNTING.**—Colony stunting is most clearly evident about 20 hours after radiation, the colony then appearing as though no growth had occurred subsequent to exposure on the radiated half, while it had proceeded normally on the non-radiated half, thus making the colony radius on this half 3-4 mm. longer than on the other half. Shortly growth is resumed by the deeper, less injured mycelium, and in a few days macroscopic evidence of the stunting is lost.

**PERITHECIAL FORMATION.**—Direct radiation, that is, that on the uncovered half of the colony, resulted in very numerous perithecia either superficial or buried, depending upon the dosage (figs. 1, 2).

Diffuse radiation, that is, the rays deflected by some irregularity of surface so that they reached under the protective shade employed, also gave abundant perithecia, either superficial or deep. Since even with very long exposures to direct radiation certain regions received very weak doses by diffusion, perithecia were produced by diffusion in exposures much too strong to give them by direct radiation.

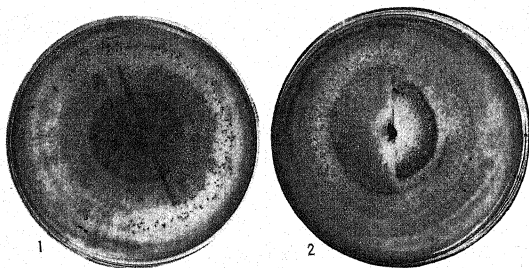
**FORMATION OF ACERVULI.**—Acervuli were distinctly more abundant in the non-radiated half of a plate following radiation of one minute through vitaglass; also short dosages of 0.25-0.75 of a second

of full radiation increased conidial production on the non-radiated portions of this colony.

#### DOSAGE

With exposures of 0.5-1 second no colony stunting was apparent; 2, 3, and 4 seconds gave slight stunting. All exposures from 5 seconds to 15 or more minutes produced distinct colony stunting.

The aerial mycelium was killed by 15 seconds. With exposures of 1-4 minutes very abnormal branching was induced, this effect increasing with the dosage; with 1.5 and 2 minutes' radiation the



FIGS. 1, 2.—Fig. 1, *G. cingulata* 8 days old radiated one-half second; numerous perithecia by direct radiation, none by diffuse radiation; fig. 2, *G. cingulata*, radiation 5 seconds; many buried perithecia by direct rays, few by diffuse rays.

stunted regions were perceptibly darkened. With exposures of 4-15 minutes the lethal effect was much greater, and numerous sclerotoid masses developed; and new centers of growth, evidently originating from deep cells that escaped the deadly rays, resulted in fan-shaped areas.

All plates exposed 1-15 minutes showed in the shielded half of the colony, close to the line of shielding, numerous perithecia. When the exposure was short (1-4 minutes) these perithecia were found very close to the shadow line, 1 mm.; given an exposure of 5-15 minutes, they were further from this line (2-3 mm.). A typical distribution is shown in fig. 2. Many of these perithecia are superficial, but some are deep in the agar (1 mm.). They vary greatly in size,

both large and small being found irregularly distributed both laterally and vertically throughout the agar.

Such perithecia are the result of rays reflected from irregularities here and there on the surface of the medium or other surroundings, and the irregularity in their size and location is due to variation in intensity of those irregularly reflected rays. The absence of perithecia close to the shadow line in heavy dosage is presumably due to a lethal intensity in this region. An occasional isolated perithecial region in the shadow, in the case of long exposure, is explained as caused by rays from some special irregularity in the reflecting surface.

A series of 4-day-old colonies was radiated with the following dosages: 1, 2, 5, 15, 30 seconds, 1, 2 minutes. The one second radiation as well as all the others induced numerous surface perithecia by diffusion. Direct radiation of 5 seconds or longer gave buried perithecia, although smaller dosage did not. The long exposures of 1 and 2 minutes gave fewer buried perithecia than did shorter exposures. Repetition with dosages of 0.5, 1, 2, 3, and 4 seconds resulted in many surface perithecia by direct radiation, mainly on the inner zone of the colony. One, 1.5, and 2 minutes gave many surface perithecia by diffusion; 1.5 and 2 minutes gave few and irregularly located deep perithecia by direct radiation.

A series of 4-day-old colonies was exposed at 230 cm. from the source of radiation for 10, 20, 30, 40, 50 seconds, 1, 1.5, 2, 5, and 10 minutes. This distance gives approximately the same energy exposure that one at 21 cm. would give in one one-hundred-twentieth of the time. Ten and 20 seconds gave negative results; 30 seconds gave surface perithecia in the oldest colony zone; 40 and 50 seconds gave the same result but intensified; 1, 1.5, 2, 5 and 10 minutes (=5 seconds at the usual distance) gave both superficial and deep perithecia.

Slight colony stunting was evident at one minute and longer at 230 cm. (=0.5 second at the usual distance), distinct stunting at 10 minutes (=5 seconds at the usual distance). Various exposures in diffused light, as from the wall, from a metal sheet, etc., gave only negative results.

The conclusions as to dosage and perithecial formation may be

summarized as follows: Direct very weak dosage gave superficial perithecia; stronger dosage gave perithecia on the surface and deeper; still stronger dosage gave only imbedded perithecia; still stronger dosage gave no perithecia. When obvious colony stunting occurs the dosage is too great for the development of surface perithecia.

#### RELATIONS OF AGAR DEPTH

Agar plates were so tilted before solidification that the agar was shallow at one side of the plate and deep at the opposite side. Four-day-old colonies on these plates were radiated for one minute. Diffuse radiation induced superficial perithecia as usual on both the deep and the shallow agar. Many buried perithecia developed in the deep agar but none in the shallow, evidently owing to over-dosage.

In a series of plates of deep agar (3 mm.), with radiation varying from 0.5 second to 5 minutes, the one-half-second direct dosage gave only surface perithecia, none by diffusion; 0.5 minute gave surface perithecia by diffusion; under direct radiation all were buried from 1 to 2 mm. deep in the agar; with 1, 2, 3, and 5 minute exposures they were buried still deeper (2-3 mm.), that is, in the lowest layer of the agar; the 3 and 5 minute dosages, however, gave but few perithecia.

#### AGE SUSCEPTIBILITY TO PERITHECIAL PRODUCTION

Colonies more than 4 days old when radiated developed perithecia in all of the exposed area, even from the youngest mycelial threads, but perithecia were often more abundant in mycelium only one day old than in older mycelium. Sometimes the one-day-old zone of the colony appeared as a dark band, due to the profusion of perithecia; in other cases the whole colony was equally perithecial. Colonies as young as 2 days old produced perithecia, but were not so satisfactory for study as were older colonies. Colonies 12 days old, on radiation, gave only a few perithecia in the 11- to 12-day-old regions, most occurring in the 5- to 9-day-old regions.

#### EFFECT OF QUALITY OF MEDIUM

Plain agar, that is, merely agar and water of the usual percentages without addition of nutrients, gave scant growth, and on radiation with various dosages gave no perithecia, probably due to lack

of sufficient vigor of growth to support perithecial formation. With the addition of corn meal agar to less than 50 per cent strength, poor growth and no perithecia resulted upon radiation. On corn meal agar of 50-70 per cent nutrient strength abundant perithecia developed on radiation.

#### POSSIBLE CHEMICAL CHANGES AS CAUSAL

To attempt to determine whether or not the radiation caused some chemical change in the medium favorable to perithecial production, two trials were made as follows: (1) A plate of sterile corn meal agar was radiated 3 minutes, then inoculated with G 10. (2) A plate of sterile corn meal agar was radiated 3 minutes, and a piece of this agar about 4×4 cm. lifted out and laid upon a 4-day-old colony covering about half of it. Growth in both cases was normal, with no perithecial development, indicating the absence under these conditions of any chemical capable of inducing perithecial formation.<sup>2</sup>

#### HEREDITY

That the sexogenetic effect is local is shown by the fact that the new mycelial growth is normal and non-sexual, although it arises directly from a mycelial region that is most strongly perithecial; that is, the perithecia are produced only on the mycelium actually stimulated, not on the progeny of these cells. Also, in numerous instances, transfers to new plates were made from regions of heavy induced perithecial formation. All such cultures behaved as did ordinary non-radiated cultures.

Two series of 8-day-old cultures were radiated for 5, 30, and 60 seconds, and were (1) inoculated from an induced perithecial region; (2) inoculated from normal non-perithecial mycelium. No difference in perithecial development was apparent between these two series, indicating that the induced ability to produce perithecia was not passed on to the progeny under the conditions of the experiment. It is to be noted, however, that in such tests as those of hereditary effects, it is possible that negative results were due to the fact that the mycelial cells that were tested, although radiated, had not responded to the stimulation. The cells actually known to have

<sup>2</sup> See also BOVIE, BOT. GAZ. 61:1. 1916; and COBLENTZ and FULTON *l.c.*, p. 663.



fig. 3. It is to be noted that with short exposures, 0.5-5 seconds, there is considerable absorption of the shorter waves, but 10-30 seconds suffices to give a very effective exposure even through this medium.

The sexogenetic effect and colony stunting were evident in all trials where Corex or vitaglass was used, but never appeared when window glass was used.

A comparative series of experiments with radiation through vitaglass and Corex for 30 seconds on colonies ranging from 2 to 8 days old showed perithecia throughout the exposed area. No essential difference between the two modes of radiation appeared, except that there was a much larger perithecial area by diffusion from Corex than from the vitaglass, due to the irregular surface of the Corex screen.

A second series with the same screens, with dosages of 10, 20, 30 seconds, 1, and 1.5 minutes showed no difference in sexogenetic effect; many buried perithecia were produced in all exposed regions of the colony. The two groups of rays do, however, produce slight differences in the contour and color of the mycelium, and consequently in the general appearance of the colony.

The conclusive evidence as to wave lengths effective in stimulating perithecial formation was given by the Hilger Quartz Spectrograph E2. To use this instrument two glass strips,  $15 \times 2.5$  cm., were placed on the bottom of a 16 cm. Petri dish 1 cm. apart, and the whole sterilized by hot air; then sufficient agar was poured in the dish to cover the glass strips to a depth of 1 mm. When solidified a row of spores was placed on the agar midway between the glass strips. This resulted, in 3 or 4 days, in a growth of susceptible mycelial threads over the entire surface of the two glass strips. These glass strips were then cut out and attached to a glass plate and placed in the plate holder. Thus, when placed in the spectrograph, the fungus growth rested exactly in the position occupied by the photographic film in ordinary exposure. The slit was adjusted at 0.75 mm. width and 1 cm. long. This gives images of the spectrum lines 1 cm. long and 1-2 mm. wide.

The first trial with an exposure of 2 hours was too long. It resulted in collapse of the aerial mycelium and in non-perithecial regions coincident with all of the more energetic bands throughout the



whole ultra-violet region down to  $180\text{ m}\mu$ , beyond which the spectrograph did not reach. By diffusion, the whole of the space between all of these bands was occupied by buried and surface perithecia.

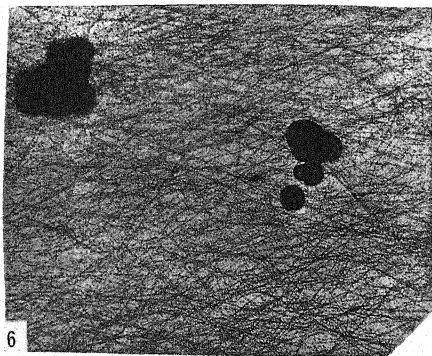
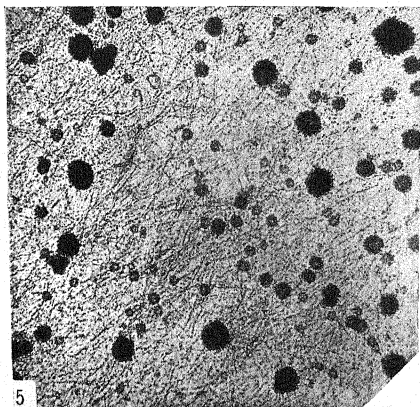
Repetition with exposures of 3, 15, 30, and 60 minutes all resulted in the death of the aerial mycelium, more pronounced with the increase of exposure, but in all cases in the development of buried perithecia throughout the whole range of the ultra-violet spectrum. Such a spectrogram printed in perithecia is shown in fig. 4. Similar trials with various separate regions of the spectrum were made. Thus the band  $254\text{ m}\mu$ , used alone, gave abundant perithecia with the 3- and 15-minute exposure, but none with 1-minute exposure. The four bands  $3021$ ,  $3023$ ,  $3026$ , and  $3027\text{ \AA}$  gave similar results. In another exposure of 15 minutes the whole range of the spectrum from  $254\text{ m}\mu$  to  $180\text{ m}\mu$  was used, resulting in perithecia throughout the range. From all the evidence, it appears that the effective rays lie throughout the region of wave lengths shorter than  $313\text{ m}\mu$ .

#### Other strains of *G. cingulata*

Of the several other strains of *G. cingulata* experimented with, nos. 2, 7, and 28 gave essentially the same results as did G 10. No. 9 behaved essentially like G 10, with the striking exception that the perithecia were produced in clumps, not scattered (figs. 5, 6, 7). No. G 9 on agar plates without radiation sometimes produced a few comparatively large (2-3 mm.) sclerotial masses on which quite numerous perithecia were borne. Nos. 8, 16, 17, and 30 produced no perithecia, but did produce acervuli on the lower, not on the upper surface of the agar, and not in the non-radiated regions (fig. 8). On no. 19 sclerotial formation was inhibited and conidial formation stimulated (fig. 9). No. 20 produced perithecia, but only in the oldest zone. Strains 4 and 19 gave only negative results as regards perithecia at 1, 15, and 60 seconds, but did produce acervuli on the mycelial tips radiated.

#### SUMMARY REGARDING *GLOMERELLA CINGULATA* UNDER FULL DIRECT RADIATION AT 21 CM.

- ✓1. Some spores are prevented from germination by exposure for 5 seconds, and all are killed or prevented from germination by 15



FIGS. 5, 6.—Fig. 5, G 10 showing scattered perithecia; fig. 6, G 9 showing perithecia in groups, not scattered.

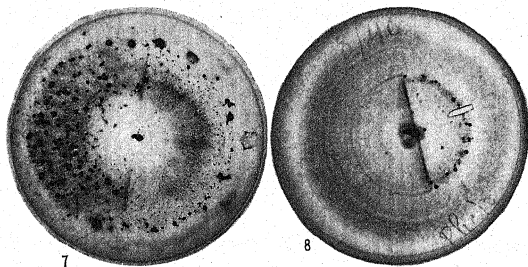
seconds' exposure; more than 90 seconds are required to kill all spores when covered by 1.5 mm. of corn meal agar.

✓2. The aerial mycelium is killed by 15 seconds' exposure.

✓3. Distinct colony stunting is produced by 5 seconds' exposure, and slight stunting by 2-4 seconds.

4. Diffuse radiation produces effects like those of much weaker direct radiation.

✓5. Direct radiation in mild dosage (0.25-4 seconds) resulted in surface perithecia; in larger dosage (5 seconds to 2 minutes) in buried perithecia.



FIGS. 7, 8.—Fig. 7, G 9, radiation 10 seconds; fig. 8, G 8, radiation 40 seconds; no perithecia but numerous acervuli in radiated portion.

✓6. With sufficient depth of agar much greater dosage may be used.

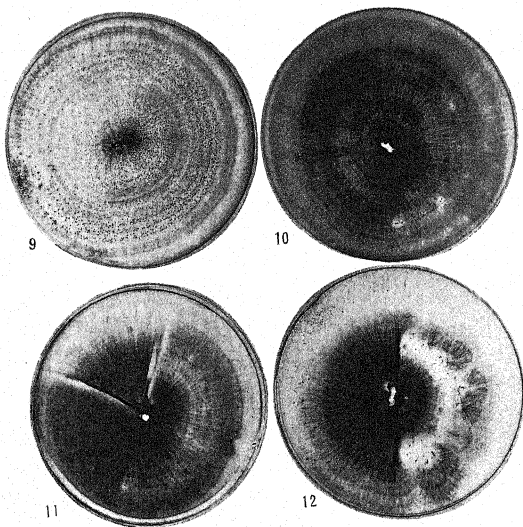
✓7. The longer the exposure the deeper in the agar lie the perithecia.

8. The rudiments of perithecia may be recognized in 15 hours, probably much earlier. The perithecia bear mature asci and ascospores in 4 days.

✓9. The youngest mycelium is most easily killed, and is most responsive in perithecial production; the older mycelium is less sensitive in both regards.

10. A medium poor in nutrients does not render a colony capable of producing perithecia on radiation.

✓ 11. The production of acervuli in the non-radiated parts of the colony is largely increased by radiation.



FIGS. 9-12.—Fig. 9, *G* 19, radiation 15 seconds; conidia produced abundantly at tips of mycelium radiated and sclerotial formation inhibited; fig. 10, *Coniothyrium*, radiation 10 seconds; many pycnidia appear at points occupied by tips of mycelial growth at time of radiation; fig. 11, *Coniothyrium*, radiation 30 seconds; many buried pycnidia in two outermost zones; fig. 12, *Coniothyrium* 8 days old, radiation 3 minutes; many buried pycnidia in oldest zone, much lethal action in youngest zone, with fanlike new growths originating from surviving centers (these centers also pycnidial).

✓ 12. There is no evidence that chemicals produced by the radiation of the medium are responsible for the effects noted. The effect is extremely local and apparently is not hereditary.

13. Different races of *G. cingulata* respond differently to radiation.

14. The efficient rays lie throughout the region of the ultra-violet of wave lengths shorter than  $313\text{ m}\mu$ .

#### *Coniothyrium* sp.

An unknown species of *Coniothyrium* which accidentally appeared upon plates in the laboratory was subjected to radiation. This fungus normally never produced pycnidia until the culture was very old, completely filling the Petri dish. It then usually, but not always, produced a circle of pycnidia at the edge of the dish.

*Coniothyrium* colonies 8 days old were radiated from 1 second to 3 minutes. With 1 second there was very slight, and at 5 seconds distinct, colony stunting but no other perceptible effect. With 10 seconds the extreme tips of the mycelium that was radiated produced numerous pycnidia, some superficial, some buried, so that a nearly perfect arc was formed by these structures (fig. 10). There were also a few scattered pycnidia throughout the zone of mycelium that was 1 to 2 days old when radiated. These were distinctly visible and well formed at 3 days, and must have been recognizable microscopically at 2 days after radiation.

With 30 and 40 seconds the result was similar, except that all pycnidia were buried and that they were more numerous in the 1- and 2-day-old region (fig. 11). With 1- and 2-minute radiation the effect differed in that some pycnidia were formed in the still older regions; with 3 minutes there was much killing of the mycelium in the 1- and 2-day-old zone, and many pycnidia were found in the oldest zone. In the youngest zone a few regions lived through the radiation and became the centers of new growth (fig. 12). The mycelium that remained alive in these centers also developed pycnidia.

From these results it appears that the youngest mycelium is most susceptible to killing and to pycnidial formation; the older less so.

#### General considerations

In both the various strains of *Glomerella cingulata* and *Coniothyrium*, radiation almost instantly initiates the development of reproductive structures in great numbers where they would not have occurred without radiation, but in no case were structures initiated that were not known to be occasionally produced by these fungi in

the natural course of events. The *Coniothyrium* normally produced but few pycnidia and these after long continued growth. On radiation they were produced at once and in quantity. Certain races of *G. cingulata* that normally produced but few perithecia and these in old colonies, on radiation produced them in great quantities at once. Other races of *G. cingulata* that have not normally been seen to produce perithecia did not yield perithecia on radiation, but even these gave a greatly increased yield of acervuli.

That ultra-violet radiation can induce deep seated changes within the cell, in chromosome number and in polarity is known.<sup>3</sup>

References to stimulation due to ultra-violet radiation below lethal dosage have been made. Thus BROWNING and RUSS<sup>4</sup> reported that bacteria lying between two regions that had been radiated appeared to be stimulated. BOVIE<sup>5</sup> states that if the decomposition of the protein molecule is not carried too far there is cell stimulation.

It appears possible that the effects noted may be due to a stimulus acting either upon the protoplasm or even upon the nuclei; or it may rest either in the production or destruction of some substance within the cell which has direct and specific effect upon cell activity. On the other hand, it is possible that these responses may be considered as in conformity to the laws of KLEBS, that a well nourished organism suddenly inhibited in growth turns to reproduction.<sup>6</sup> However, it is to be noted that while this particular stimulus by definite bands of rays calls forth these responses, no other known agency causing the cessation of growth (and many such have been tested) does call forth these responses. Moreover, the effect is almost if not quite immediate, which makes it difficult to regard the phenomenon as merely a reaction to inhibited growth. Furthermore the action is sharply localized, only the cells impinged upon and not all of these respond. If the cause rested merely upon starvation the results would presumably be more widely distributed among the adjacent mycelial cells.

<sup>3</sup> See JUST as cited by W. C. CURTIS, Science N.S. 67:141-149. 1928.

<sup>4</sup> Archives Radiology and Electrotherapy 18:85. 1918.

<sup>5</sup> BOVIE, I.C. See also COBLENTZ, W. W., and FULTON, H. R., Sci. Pap. Bur. Standards. no. 495. 1924 (p. 647).

<sup>6</sup> Jahr. Wiss. Bot. 53:72-73. 1900.

It is of interest to note that there is practically no solar radiation at sea level of less wave length than  $305\text{ m}\mu$  transmitted through the atmosphere; therefore these fungi in the course of their evolution have not had to contend with or adapt themselves to such influences, although they have had to do so as regards wave lengths longer than  $305\text{ m}\mu$ .

In addition to any theoretical interests that these phenomena may have, their discovery also serves a practical scientific end, in that by means of ultra-violet radiation we now have a means of inducing largely increased sexual, pycnidial, or other conidial formation; and we can produce either perithecia (*Glomerella*) or pycnidia (*Coniothyrium*) under such conditions that the age of these structures may be definitely known, thus rendering cytological and morphological studies much more definite and trustworthy.

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## SYMBIOSIS IN A DECIDUOUS FOREST

### III. MYCORRHIZAL RELATIONS

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The deciduous forest in which these symbiosis studies are being made is known as the University Woods, and is located near Urbana, Illinois. It was described in the first paper of the series.<sup>1</sup> According to the counts there reported, the forest contains 183 species of seed plants. We have examined the roots of 145 of these species, or approximately 80 per cent, to determine the presence or absence of mycorrhizal fungi. The method used has been to collect pieces of the smaller roots from two individuals of each species. The roots from each individual were separated into two lots, imbedded, sectioned, and two slides made from each lot. In all cases some sections were cut near the root tip and others in the region of cell maturation. We thus have eight slides from each species, each slide bearing sections from one, two, or three roots, making a total of nearly 1200 slides. The slides were then examined microscopically for mycorrhizal fungi. In many cases a binocular microscope with oil immersion lens was necessary to reach a decision, but both writers have examined all slides and agreed in the observations. The results of the observations follow, the nomenclature and arrangement into families being that of GRAY's *New manual of botany*, 7th ed.

#### GRAMINEAE:

*Elymus striatus*, endotrophic mycorrhizas.

*Hystrix patula*, no fungus observed.

*Poa sylvestris*, no fungus observed.

#### CYPERACEAE:

*Carex grayii*, no fungus observed.

#### ARACEAE:

*Arisaema dracontium*, endotrophic mycorrhizas.

*Arisaema triphyllum*, no fungus observed.

#### COMMELINACEAE:

*Tradescantia pilosa*, endotrophic; fungus not abundant.

<sup>1</sup> BOT. GAZ. 73:200-212. 1922.



## LILIACEAE:

*Polygonatum commutatum*, endotrophic; fungus scarce and often disintegrated.

*Smilax herbacea*, endotrophic; fungus scarce.

*Smilax hispida*, no fungus observed.

*Smilicina racemosa*, endotrophic mycorrhizas.

*Trillium recurvatum*, no fungus observed.

*Uvularia perfoliata*, no fungus observed.

## IRIDACEAE:

*Iris hexagona*, endotrophic; fungus not abundant.

## ORCHIDACEAE:

*Aplectrum hyemale*, endotrophic; in addition to usual endophyte there is present in some roots a coarse, septate mycelium showing clamp connections; probably a casual parasite.

## SALICACEAE:

*Populus deltoides*, ectotrophic mycorrhizas.

## JUGLANDACEAE:

*Carya cordiformis*, some endotrophic and some ectotrophic mycorrhizas.

*Carya laciniosa*, endotrophic; some roots contain a septate mycelium similar to that observed in *Aplectrum hyemale*.

*Juglans cinerea*, endotrophic; vesicules observed.

*Juglans nigra*, endotrophic; fungus scarce and much disintegrated.

## BETULACEAE:

*Carpinus caroliniana*, ectotrophic mycorrhizas.

## FAGACEAE:

*Quercus imbricaria*, ectotrophic mycorrhizas.

*Quercus macrocarpa*, ectotrophic mycorrhizas.

*Quercus muhlenbergii*, ectotrophic mycorrhizas.

*Quercus rubra*, ectotrophic mycorrhizas.

## URTICACEAE:

*Celtis occidentalis*, endotrophic; fungus rather indistinct.

*Humulus lupulus*, endotrophic; a septate mycelium similar to that observed in *Aplectrum* is present in addition to the usual endophyte.

*Laportea canadensis*, no fungus observed.

*Morus rubra*, endotrophic mycorrhizas.

*Pilea pumila*, endotrophic mycorrhizas.

*Ulmus americana*, no fungus observed.

*Ulmus fulva*, endotrophic mycorrhizas.

ARISTOLOCHIACEAE:

*Asarum canadense*, endotrophic mycorrhizas.

POLYGONACEAE:

*Polygonum scandens*, no fungus observed.

*Polygonum virginiana*, no fungus observed.

*Rumex crispus*, no fungus observed.

PHYTOLACCACEAE:

*Phytolacca decandra*, no fungus observed.

PORTULACACEAE:

*Claytonia virginica*, no fungus observed.

RANUNCULACEAE:

*Actaea alba*, no fungus observed.

*Anemone canadensis*, endotrophic; fungus scarce.

*Hepatica triloba*, endotrophic mycorrhizas.

*Isopyrum bilernatum*, no fungus observed.

*Ranunculus septentrionalis*, no fungus observed.

*Thalictrum dioicum*, endotrophic mycorrhizas.

ANONACEAE:

*Asimina triloba*, endotrophic mycorrhizas.

MENISPERMACEAE:

*Menispermum canadense*, no fungus observed.

BERBERIDACEAE:

*Caulophyllum thalictroides*, no fungus observed.

*Podophyllum peltatum*, endotrophic; vesicles present.

LAURACEAE:

*Benzoin melissaeifolium*, no fungus observed.

PAPAVERACEAE:

*Sanguinaria canadensis*, no fungus observed.

FUMARIACEAE:

*Dicentra canadensis*, no fungus observed.

*Dicentra cucullaria*, no fungus observed.

CRUCIFERAE:

*Cardamine douglassii*, no fungus observed.

*Dentaria laciniata*, no fungus observed.

## PLATANACEAE:

*Platanus occidentalis*, endotrophic mycorrhizas.

## ROSACEAE:

*Agrimonia mollis*, endotrophic; vesicules present.

*Crataegus crus-galli*, endotrophic mycorrhizas.

*Crataegus mollis*, endotrophic mycorrhizas.

*Geum canadense*, endotrophic; fungus not abundant.

*Prunus serotina*, endotrophic mycorrhizas.

*Rubus occidentalis*, endotrophic mycorrhizas.

## LEGUMINOSAE:

*Cercis canadensis*, endotrophic; one root shows perithecium containing large spiny two-celled spores.

*Desmodium grandiflorum*, endotrophic; fungus much disintegrated.

*Gleditsia triacanthos*, endotrophic mycorrhizas.

*Gymnocladus dioica*, no fungus observed.

## RUTACEAE:

*Zanthoxylum americanum*, no fungus observed.

## EUPHORBIACEAE:

*Acalypha virginica*, endotrophic; fungus scarce.

## LIMNANTHACEAE:

*Floerkea proserpinacoides*, no fungus observed.

## CELASTRACEAE:

*Celastrus scandens*, endotrophic mycorrhizas.

*Evonymus atropurpureus*, endotrophic mycorrhizas.

## ANACARDIACEAE:

*Rhus toxicodendron*, endotrophic mycorrhizas.

## STAPHYLEACEAE:

*Staphylea trifolia*, endotrophic mycorrhizas.

## ACERACEAE:

*Acer negundo*, endotrophic mycorrhizas.

*Acer saccharum*, endotrophic mycorrhizas.

*Acer saccharinum*, endotrophic mycorrhizas.

## SAPINDACEAE:

*Aesculus glabra*, endotrophic mycorrhizas.

## BALSAMINACEAE:

*Impatiens biflora*, endotrophic mycorrhizas.

*Impatiens pallida*, endotrophic mycorrhizas.

## VITACEAE:

*Psedera quinquefolia*, endotrophic mycorrhizas.

*Vitis cordifolia*, endotrophic mycorrhizas.

## TILIACEAE:

*Tilia americana*, ectotrophic mycorrhizas.

## VIOLACEAE:

*Viola affinis*, endotrophic; fungus scarce.

*Viola scabriuscula*, endotrophic; vesicles present.

## ONAGRACEAE:

*Circaea intermedia*, no fungus observed.

*Oenothera biennis*, no fungus observed.

## ARALIACEAE:

*Panax quinquefolia*, endotrophic mycorrhizas.

## UMBELLIFERAE:

*Cryptotaenia canadensis*, endotrophic mycorrhizas.

*Osmorhiza claytoni*, no fungus observed.

*Osmorhiza longistylis*, endotrophic mycorrhizas.

*Sanicula canadensis*, endotrophic mycorrhizas.

## CORNACEAE:

*Cornus stolonifera*, endotrophic mycorrhizas.

## PRIMULACEAE:

*Steironema ciliatum*, endotrophic; fungus scarce.

## OLEACEAE:

*Fraxinus americana*, endotrophic mycorrhizas.

*Fraxinus pennsylvanica lanceolata*, endotrophic mycorrhizas.

*Fraxinus quadrangulata*, endotrophic; fungus scarce and much disintegrated.

## ASCLEPIADACEAE:

*Asclepias syriaca*, endotrophic mycorrhizas.

## POLEMONIACEAE:

*Phlox divaricata*, no fungus observed.

## HYDROPHYLLACEAE:

*Hydrophyllum appendiculatum*, no fungus observed.

*Hydrophyllum canadense*, no fungus observed.

*Hydrophyllum virginianum*, no fungus observed.

## BORAGINACEAE:

*Lappula virginiana*, no fungus observed.

*Mertensia virginica*, no fungus observed.

## VERBENACEAE:

*Verbena urticaefolia*, endotrophic; vesicules present.

## LABIATAE:

*Agastache nepetoides*, endotrophic; fungus scarce.

*Blephilia hirsuta*, endotrophic; fungus disintegrated.

*Leonurus cardiaca*, no fungus observed.

*Nepeta cataria*, no fungus observed.

*Nepeta hederacea*, no fungus observed.

*Prunella vulgaris*, no fungus observed.

## SOLANACEAE:

*Solanum carolinense*, no fungus observed.

## SCROPHULARIACEAE:

*Collinsia verna*, no fungus observed.

*Scrophularia marilandica*, endotrophic; fungus disintegrated and not abundant.

*Verbascum thapsus*, no fungus observed.

*Veronica virginica*, no fungus observed.

## BIGNONIACEAE:

*Tecoma radicans*, endotrophic mycorrhizas.

## ACANTHACEAE:

*Ruellia strepens*, endotrophic mycorrhizas.

## PHRYMACEAE:

*Phryma leptostachya*, no fungus observed.

## RUBIACEAE:

*Galium aparine*, no fungus observed; many cortical cells contain coccus-like bacteria.

*Galium concinnum*, endotrophic; fungus very scarce.

## CAPRIFOLIACEAE:

*Sambucus canadensis*, endotrophic mycorrhizas.

## CAMPANULACEAE:

*Campanula americana*, endotrophic mycorrhizas; vesicules present.

## LOBELIACEAE:

*Lobelia inflata*, endotrophic; fungus very scarce.

*Lobelia siphilitica*, no fungus observed.

## COMPOSITAE:

*Actinomeris alternifolia*, endotrophic mycorrhizas.

*Ambrosia artemisiifolia*, endotrophic mycorrhizas.

*Ambrosia trifida*, endotrophic mycorrhizas.

- Aster ericoides*, endotrophic mycorrhizas.  
*Aster sagittifolius*, endotrophic; much of the fungus disintegrated.  
*Aster shortii*, endotrophic; fungus very scarce.  
*Bidens vulgata*, endotrophic mycorrhizas.  
*Cacalia reniformis*, endotrophic; fungus very scarce.  
*Erigeron canadensis*, no fungus observed.  
*Erigeron philadelphicus*, no fungus observed.  
*Eupatorium purpureum*, endotrophic; fungus disintegrated.  
*Eupatorium urticaefolium*, no fungus observed.  
*Helianthus decapetalus*, endotrophic mycorrhizas.  
*Lactuca floridana*, endotrophic; fungus disintegrated.  
*Lactuca scariola*, endotrophic; fungus very scarce.  
*Polymnia canadensis*, no fungus observed.  
*Rudbeckia laciniata*, endotrophic mycorrhizas.  
*Rudbeckia triloba*, endotrophic mycorrhizas.  
*Solidago rugosa*, endotrophic mycorrhizas.  
*Taraxacum officinale*, endotrophic mycorrhizas.  
*Vernonia illinoensis*, endotrophic; fungus disintegrated.

This list contains 145 species distributed among 114 genera and 60 families. Mycorrhizal fungi were found in 93 species, 76 genera, and 43 families; while in 52 species, 38 genera, and 17 families no mycorrhizal fungi were observed. In other words, either ectotrophic or endotrophic mycorrhizas were found in 64.1 per cent of the species, 66.6 per cent of the genera, and 71.6 per cent of the families. It is quite possible if not probable, of course, that more extensive observations would materially decrease the percentage of negative results. Perhaps the most significant fact brought out by these observations, however, is the large percentage of plants in this forest that harbor mycorrhizal fungi, which reemphasizes the great importance of a clearer understanding of mycorrhizal phenomena than has heretofore been possible.

Of the 93 species that were found to possess mycorrhizas, only 8 (all of which are trees) were ectotrophic; while 86 were endotrophic, *Carya cordiformis* showing both mycorrhizal types. Endotrophic mycorrhizas in species of *Carya* are here reported for the first time, although the ectotrophic type is common in this genus and the endotrophic type is well known in the closely related genus *Juglans*.

Apparently there are very few woody plants that do not at times harbor mycorrhizal fungi of one or more kinds. The only woody plants in this forest on which no mycorrhizas were found are *Ulmus americana*, *Benzoin melissaefolium*, *Gymnocladus dioica*, and *Zanthoxylum americanum*; and one of these, *Ulmus americana*, has been found with endotrophic mycorrhizas in other habitats. It is interesting to note, too, that in some of the larger families, such as Rosaceae and Compositae, mycorrhizas are common; while in Labiatae and Scrophulariaceae they are relatively uncommon.

Nearly all of the endophytic fungi observed in this study appear to be phycomycetous. Basidiomycetous mycelia showing septa and clamp connections were found in *Aplectrum hyemale*, *Carya laciniosa*, and *Humulus lupulus*; but in all cases they appeared to be casual parasitic invaders rather than true mycorrhizal fungi. Entirely comparable with these basidiomycetous parasites would be the bacteria that were observed in the cortical cells of *Galium aparine* and the perithecia-forming fungus in one root of *Cercis canadensis*.

In the case of practically every endotrophic mycorrhizal plant here reported, nothing definite is known concerning its mycorrhizal relations except that it has mycorrhizas. They thus furnish a very fertile field for investigation. In some the endotrophic fungus is rare while in others it is practically always present and usually abundant. In the maples, for example, the fungus is almost always to be found in the cortical cells of beadlike swellings of the small rootlets. It is hoped that opportunity for further investigation of this particular genus may be forthcoming.

At present there is absolutely no evidence that any of the higher plants here considered are benefited by the presence of mycorrhizal fungi. It is possible that in some of these cases the relation between fungus and higher plant is reciprocal, as it has been found to be in the orchid family and in some members of the heath family; but up to the present we have no basis for believing that this is true. In many cases the fungus mycelium appears much disintegrated, but in no case has evidence of the actual digestion of fungus filaments by the host cells been seen, as reported in the preceding families; and no attempts have yet been made to determine whether seedlings of any of these plants may be grown under sterile conditions in the

absence of fungi. On the other hand, it is well known that the aerial parts of plants are subjected to the attacks of a variety of relatively harmless parasites, such as gall-forming insects, leaf-spot fungi, etc., and there is no reason for supposing that the subterranean parts are exempt from similar attacks. For the present, therefore, it is believed that all of the mycorrhizas reported in this paper should be classified under antagonistic nutritive conjunctive symbiosis, the fungus being parasitic on the higher plant.

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## EFFECT OF ALCOHOL ON CELLS OF NITELLA FLEXILIS

P. A. DAVIES

(WITH ONE FIGURE)

### Introduction

An initial rise in the rate of  $\text{CO}_2$  production<sup>1</sup> is induced by the action of ethyl alcohol on cells of *Nitella flexilis*. Such a rise has been observed by others using different substances. IRVING (7) observed a rise when young shoots of *Hordeum vulgare* were subjected to low concentrations of chloroform, but with high concentrations no rise occurred. HAAS (4, 5, 6), using alcohol, acetone, formaldehyde, and ethyl chloride on *Laminaria* tissue, and GUSTAFSON (3), using formaldehyde, ether, and acetone on *Aspergillus niger*, found that when the concentrations were high enough to produce an effect, there was an increase, followed by a decrease, in the rate of  $\text{CO}_2$  production. HAAS (4, 5) found the rate to be higher than the normal even after the tissue was killed. BROOKS (1) found an enormous rise (50 times the normal) when *Bacillus subtilis* was treated with 7.3 per cent ether solution, but no rise with high concentrations. RAY (13) found that subjecting *Ulva* tissue to 0.25 per cent chloroform solution gave an initial rise, but above 0.5 per cent only a decrease from the normal was observed. The writer's results with alcohol are very similar to those of HAAS and GUSTAFSON, but differ from those of IRVING, BROOKS, and RAY, in that an initial rise occurred with all concentrations used.

This preliminary paper is concerned with a brief explanation of the cause of the relative heights of the initial rises. In all experiments a normal rate of  $\text{CO}_2$  production was established (taken as 100 per cent) before the alcohol was added.

### Discussion of results

Fig. 1 shows the relative heights in the initial rate of  $\text{CO}_2$  production induced by the action of different concentration of alcohol.

<sup>1</sup> For apparatus used in  $\text{CO}_2$  determinations, see OSTERHOUT (11). All experiments were conducted at 30° C.

Comparing the initial rises, the 20 per cent (by volume) solution caused the highest initial rise, followed by the 10, 30, 40, 60, and 95 per cent solutions respectively. The drop of the initial rises below the normal rate was most rapid in the 95 per cent solutions, followed by the 60, 40, 30, 20, and 10 per cent solutions respectively; al-

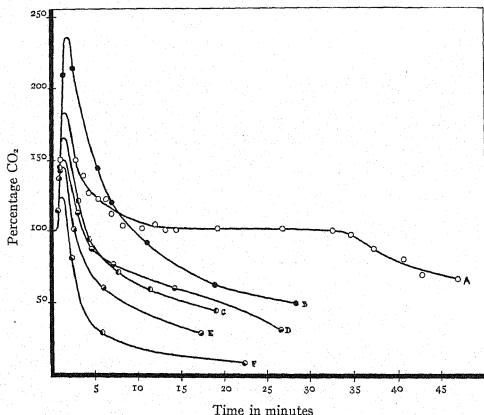


FIG. 1.—Rate of CO<sub>2</sub> production from cells of *Nitella flexilis* in different concentrations of ethyl alcohol, as percentages of normal rates: A, effect of 10 per cent (by volume) solution; B, 20 per cent; C, 30 per cent; D, 40 per cent; E, 60 per cent; F, 95 per cent. Each curve represents a single experiment best illustrating the average course of all (3 to 13) experiments.

though in the 10 per cent solution the rate of CO<sub>2</sub> production did not fall below the normal rate until about 35 minutes after the solution was added.

The question might arise as to whether the relative increases may not be due to the coefficient of CO<sub>2</sub> absorption by the different concentrations of alcohol. If the coefficient of CO<sub>2</sub> absorption by alcohol alone is considered, we should expect to find the highest initial rise in a concentration greater than 20 per cent. MÜLLER (10) found the

lowest coefficient of  $\text{CO}_2$  absorption by alcohol at approximately  $20^\circ \text{C}$ . in a solution of 28.46 per cent by weight (36.25 per cent by volume). LUBARSCH (9) confirmed MÜLLER's results by finding the lowest coefficient of  $\text{CO}_2$  absorption in a 28.5 per cent solution; so it appears that the highest initial rate obtained in the 20 per cent solution is independent of the  $\text{CO}_2$  absorption by the alcohol, and must be due to the direct action of the alcohol on the structures of the cell.

As to the source of the  $\text{CO}_2$ , BROOKS believes that the probable source is  $\text{CO}_2$  previously stored in the cell, either as  $\text{CO}_2$  or in the form of carbonates or bicarbonates. The writer's work seems to show that the chief source of  $\text{CO}_2$  is  $\text{CO}_2$  dissolved, under tension, in the cell sap and not in the form of carbonates or bicarbonates. When extracted cell sap of *Nitella*<sup>2</sup> was used an initial rise was secured when distilled water was added. When new extractions of the cell sap were taken and  $\text{CO}_2$ -free air allowed to bubble through them for 10 minutes, when tested with distilled water or dilute hydrochloric acid, no initial rise was secured; in fact no measurable amount of  $\text{CO}_2$  was secured. In order to check these results, potato sap known to contain dissolved  $\text{CO}_2$  was tested, and similar results were obtained. The effect of the distilled water must be on the  $\text{CO}_2$  equilibrium of the extracted saps. That  $\text{CO}_2$  is held in the cell sap under considerable tension is shown by CROZIER (2) and by OSTERHOUT and DORCAS (12) with cells of *Valonia macrophysa*. They found that the sap is a poor buffer, CROZIER finding that 1-2 cc. of sea water was sufficient to change 10 cc. of the sap from pH 5.9 (average pH of the cell sap) to 7.0. IRWIN (8) gives the pH value of *Nitella* sap as 5.6; a greater internal  $\text{C}_\text{H}$  than was found for *Valonia*. The sudden increase in  $\text{CO}_2$  production when distilled water was added to *Nitella* sap, with a rapid fall below the normal, and the fact that no production of  $\text{CO}_2$  can be observed after  $\text{CO}_2$ -free air has been bubbled through the cell sap for 10 minutes, indicate, as has been shown for *Valonia*, that the pH value of the sap must be due chiefly to  $\text{CO}_2$  dissolved in it. RAY thinks that the initial rise is due to the fact that the reagent (chloroform) reacts directly with the oxidase

<sup>2</sup> The cells were washed several times in distilled water and squeezed between several layers of neutralized and sterilized cheesecloth, and the sap was placed in the apparatus in an unfiltered condition.

system, either as a catalyzer or by the formation of a loose compound with some portion of this system. The writer obtained an initial rise when normal cells of *Nitella* were treated with distilled water, as when (as has already been explained) distilled water was added to the extracted saps of *Nitella* and potato. It is hard to believe that water could produce such a rapid production of  $\text{CO}_2$  by catalysis, for in the case of potato sap it was diluted with distilled water before being placed in the apparatus. The action of the water on the saps must be due simply to the upsetting of the  $\text{CO}_2$  equilibrium to such an extent that  $\text{CO}_2$  is released. The action of distilled water on the normal cells must be such as to produce a sudden change in the plasma membranes, so that  $\text{CO}_2$ , where it is held under considerable tension, is rapidly given off for a short time.

The action of the alcohol depends on its concentration: in the 10 per cent solution it did not penetrate<sup>3</sup> the cell structures rapidly enough to cause a rapid release of  $\text{CO}_2$  induced by the 20 per cent solution; in the 30, 40, 60, and 95 per cent solutions the alcohol caused a change of the cell structures to such an extent that  $\text{CO}_2$  was released slowly from the irreversibly injured cells and was taken up by the solution, as it escaped, according to the coefficient of  $\text{CO}_2$  absorption by the solutions; and in the 20 per cent solution the alcohol caused an intermediate effect, a rapid penetration without a rapid change of the cell structures, allowing a more rapid outflow of  $\text{CO}_2$ .

### Summary

1. The relative rises in the initial rates of  $\text{CO}_2$  production are due to the direct action of the different concentrations of ethyl alcohol on the complex structures of the cell, which control the internal  $\text{CO}_2$  tension.

2. The 20 per cent (by volume) solution caused the highest initial rise, followed by the 10, 30, 40, 60, and 95 per cent solutions respectively.

3. The drop of the initial rises below the normal rates was most rapid in the 95 per cent solution, followed by the 60, 40, 30, 20, and 10 per cent solutions respectively.

<sup>3</sup> Irreversible injury, considered to have taken place when the rigidity of the cell was lost, did not occur until about 33 minutes and 58 seconds (average time) after the alcohol was added.

4. The highest initial rise in the 20 per cent solution is produced by a rapid penetration of the alcohol without a rapid change in the cell structure, allowing a rapid outflow of  $\text{CO}_2$ .

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## BRIEFER ARTICLES

### THE MULTIPLE-SEEDED XANTHIUM

CHARLES A. SHULL

(WITH ONE FIGURE)

The discovery of another native specimen of the multiple-seeded *Xanthium*, which is tentatively known as *Xanthium chinense globuliforme*, in a locality far removed from the previously recorded localities for this form, emphasizes the sporadic occurrence of this peculiar type of cocklebur. This specimen, which is illustrated in fig. 1, was found by Mr. GEO. M. CHASE, who lives about 6 miles southeast of Allen, Nebraska, a short distance west of Sioux City. The plant was found growing in a cornfield, among many other specimens of *Xanthium* and *Helianthus*. It attracted attention because it was so different from its surrounding plants, and was sent to Professor W. W. BURR of the University of Nebraska for identification. Through the kindness of Professors BURR and N. F. PETERSON, who recognized its similarity to the previously reported multiple-seeded forms, it was sent to the writer for examination.

The photograph is too small to show the characteristic structure of the bur, unless examined with a hand lens; but it is quite similar to that of those previously described.<sup>1,2,3</sup> This particular plant is over 4 feet tall, and shows evidence of great vegetative vigor. It is completely sterile. The burs are short and rather deeply cleft. While a few shriveled ovaries can be found in the largest burs, none of them contains even partially developed embryos. The stem is very strongly fasciated, as is readily seen in the figure. In its tendency to complete sterility, in its vegetative vigor, in its strongly fasciated stem, it resembles many of the specimens of multiple-seeded *Xanthium* which have been grown from seed in the garden of the Hull Botanical Laboratory.

In a recent paper<sup>3</sup> the various possible interpretations of this

<sup>1</sup> SHULL, C. A., An interesting modification in *Xanthium*. Amer. Jour. Bot. 3:40-43. 1917.

<sup>2</sup> ———, Multiple-seeded burs of *Xanthium*. Science 58:145-146. 1923.

<sup>3</sup> ———, Nature of the multiple-seeded *Xanthium*. BOT. GAZ. 83:385-398. 1927.

type of *Xanthium* have been summarized. Reversion to remote ancestry, mutation, fasciation, and hybridization are the main possibilities. Similar "reversions" have been reported among other genera of the Compositae, and fasciation is rather readily induced in this family. After careful study of the behavior of the plants through a number of generations, it seemed more likely that the freak multiple-seeded condition is a consequence of fasciation, rather than of hybridization or reversion. Yet there are a number of facts which deserve consideration.

In the second generation offspring of the specimen found in Kansas City in 1925, there was great variability in size. There was a suggestion of segregation in the differences noted in the size and vigor of growth of specimens during the summer of 1927. Yet there has never been any clear cut segregation in regard to bur character, such as one might expect if the multiple-seeded forms are hybrids. As these specimens were started under rather poor greenhouse conditions, which may have affected their vigor after transplanting to the garden, the experiment is to be repeated with seeds planted in the garden at the normal season of germination.

An interesting observation has been made by Miss SYMONS,<sup>4</sup> which is worthy of serious consideration. She reports cases of bur character segregations, as for instance, burs of *X. pennsylvanicum* giving in the next generation both *chinense* and *pennsylvanicum*. She describes these burs very briefly, as follows:

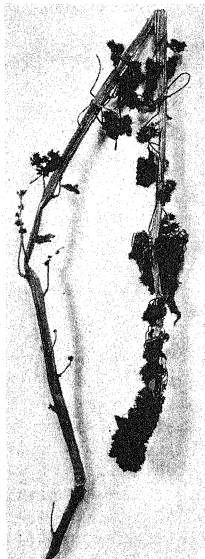


FIG. 1.—Sterile native specimen of multiple-seeded *Xanthium*

<sup>4</sup> SYMONS, JENNIE L., Studies in the genus *Xanthium*. BOT. GAZ. 82:121-147. 1926.

Their fruits were small, with few prickles, and characteristic wide open beaks, which were purple-tipped when immature. The latter, usually indicative of the number of seeds in the bur, varied in number from 2 to 7. The formation of more than the normal number of beaks and seeds was especially characteristic of these *X. chinense* plants, although it occurred to some extent in all plants of this lot.

This is the only evidence we have that seems to link multiple-seededness with hybridization.

Miss SYMONS says nothing about the bearing which this observation has upon the nature of the naturally occurring multiple-seeded specimens, but it is very suggestive, and deserves much careful study. She proceeded to cross various species, particularly *X. italicum* and *X. curvescens* with each other, and with *X. inflexum* and *X. pennsylvanicum*, and demonstrated the possibility of such crosses. Her tests of the possibility of parthenogenesis agree with some results obtained by the writer at Johns Hopkins University in 1923. There is apparently no parthenogenesis, and cross fertilization is not abundant between specimens isolated by a distance of 50 feet or more from other plants of the same species.

Examination of the burs produced by the hybrids obtained by Miss SYMONS fails to reveal anything like the burs which have been found on these multiple-seeded specimens found occurring sporadically in nature. Whether succeeding generations from these hybrids would show the same type of multiple-seeded condition is problematical; but the fact that some multiple-seeded specimens of *X. chinense* were observed in her studies, whether of the same type as the native burs or not, should lead to a thorough testing of species crosses, to determine whether multiple-seeded burs like those in nature can be produced by crossing the proper species. Granting the possibility that these sporadic multiple-seeded forms may be due to rare hybridizations, yet we have never noted any segregation of bur types in successive generations, and fasciation is practically universal in the native specimens and their offspring. This has not been reported to be the case with the crosses so far achieved, but should be looked for in all cases of known hybrids. The phenomena may be restricted to certain species crosses, and fail to develop in other cases of hybridization.

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# CURRENT LITERATURE

## BOOK REVIEWS

### Leaf miners

A volume has been prepared by NEEDHAM, FROST, and TOTHILL<sup>1</sup> which will be of interest and use to botanists, to lovers of plants, and to plant pathologists as well as to entomologists.

Chapters I and II are of general biological interest, and afford pleasant reading. Phrases are used in places for which a botanist would take a fellow botanist to task; possibly the entomologist should be given greater freedom. There is slight justification for referring to leaf vessels as "channels of circulation," however, or for grouping everything between the lower and upper epidermis, veins included, as "mesophyll." Various orders and families of insects which play a rôle as leaf miners are discussed in the following twelve chapters. Chapters XV and XVI will prove especially welcome to anyone not a professional entomologist. The former consists of a list of leaf-mining insects, while the latter contains a list of hosts of leaf miners and an extensive bibliography. In this list plants are given under both scientific and common names, when they have the latter. There are listed 422 hosts. For any one primarily interested in plants, this list is the most important feature of the book.—G. K. K. LINK.

### Truck-crop plants

A book has been prepared by JONES and ROSA<sup>2</sup> which will serve as a very general reference volume for those interested in the plants which are classed as truck-crops. There is a brief discussion of the taxonomy, histology, morphology, physiology, ecology, pathology of non-parasitic and parasitic origin, and genetics of these plants. In addition, some attention is devoted to the breeding, cultural, and marketing practices associated with these crops. In presenting these data, the authors have succeeded in their task of preparing a survey of present information on truck-crop plants in which the plant rather than "certain practices or phenomena" is made the basis for discussion.—G. K. K. LINK.

## NOTES FOR STUDENTS

**Photosynthesis.**—DANGEARD<sup>3</sup> has recently devoted a whole volume of his botanical journal to the publication of the results of his work on photosynthesis scattered over some twenty years. Ignored by his compatriots, although

<sup>1</sup> NEEDHAM, J. G., FROST, S. F., and TOTHILL, BEATRICE H., Leaf mining insects. 8vo. viii+351. figs. 91. Baltimore: Williams and Wilkins Co., 1928.

<sup>2</sup> JONES, H. A., and ROSA, J. T., Truck-crop plants. 8vo. xiv+538. figs. 98. New York: McGraw Hill Book Co. 1928.

<sup>3</sup> DANGEARD, P. A., Recherches sur l'assimilation chlorophyllienne et les questions qui s'y rattachent. *Botaniste* 19(1/6):1-397. 1928.

doing the best work in this field which France has produced, it is not surprising that his name is little known as a student of this problem. His papers hitherto have been rather brief notes of progress, often in somewhat obscure journals and always with the irritating lack of details so customary in French scientific papers. Even the two recent exhaustive monographs on photosynthesis by STILES and by SPOEHR fail to make any reference to DANGEARD's work, although it is cited in several of the papers they review. Because of this curious neglect, and because the work reported seems unusually interesting, it is called to the attention of plant physiologists in some detail.

It is unfortunate that most of the work now reported was done before the war, although it has been confirmed by recent repetition. Had it been published in full then, it would undoubtedly have been acclaimed loudly; now much of his work has been better done by others, especially as regards quantitative effects of light. But DANGEARD has studied particularly the qualitative effects, and has much that is still worth reading. His "new method," published in 1909, is still unadopted by anyone else. It is based on the use of cultures of non-motile, unicellular green algae, especially *Chlorella* and *Scenedesmus*. These algae in an inorganic nutrient medium are quite unable to multiply appreciably in the dark, whereas in the light they multiply rapidly. Such a culture, placed behind a series of ray filters or in a spectrum, will cover the vessel wall in regions where photosynthesis can take place, but fail to multiply and become visible in regions where it cannot occur. Furthermore, such cultures give off bubbles of oxygen which are readily counted (1-400 per m.), and the rate of bubble production is a much safer criterion of photosynthetic activity than in the case of *Elodea*. There is no inactive tissue and no internal aerating system to hold nitrogen, as in *Elodea*, and changes in light intensity are followed very quickly by changes in bubble rate, so that a new equilibrium is established in 1-3 minutes. These algal cultures are easily maintained in quantity, and cultures giving comparable results readily selected, so that the material can be standardized. Although WARBURG has used these algae for his quantitative studies of photosynthetic efficiency, nobody but DANGEARD has used them for the ordinary studies (and laboratory experiments!) on photosynthesis. The reviewer believes that here is a tool to our hand which may give valuable service.

The historical introduction covers 63 pages, and is a well tempered but penetrating critique of the work up to date, especially with respect to the qualitative effect of light. The only omission noted is of the work of WARBURG and NEGELEIN, which did not appear in a botanical journal. Particular attention is given to the errors involved in studies using ray filters, such as  $\text{CuSO}_4$  solution, and the necessity pointed out of determining spectroscopically the wave lengths which pass. WARBURG and NEGELEIN did this, and tried to correct for undesired transmission; but most workers have failed to note the parasitic radiations. The fallacy of WURMSER's well known and oft criticized anomalous results is declared to be due to his green filter passing red rays of great photosynthetic activity.

The author has attacked the problem of the relation between wave length and photosynthesis with both ray filters and spectra. The Wratten series of seven monochromatic gelatin filters was used principally for the first method, and each filter was analyzed with the spectroscope before and after use, as they were found to change in range of transmission with use. For spectral work three glass prism and one quartz system spectrographs were employed. The latter gave an approximately normal spectrum of considerable intensity throughout, dispersion in the violet being less than twice that in the red. A 1 mm. slit was used. The light source for spectral work was usually an electric filament lamp.

Whether with ray filters or with spectra, there was algal growth and bubble production only at the position of the absorption bands of chlorophyll, and the amount of growth or bubbles was proportional to the degree of absorption. The green covering on the walls of the culture used in the spectrum was in bands agreeing with the absorption bands. This was true of all absorption bands save the very strong ones in the blue-violet, where neither growth nor bubble production was appreciable, even after several days' illumination. Using the filters with very intense light, as direct insolation, there might be considerable activity in regions not ordinarily active, as in green and blue-violet, and the author produces considerable evidence for his view that this is almost wholly accounted for by the parasitic red rays passed then. It is unfortunate that no measurements were made of the energy distribution of the spectrum of the electric lamp used, but sunlight studies confirmed those with the artificial light source. Evidence is presented that multiplication of algae in the B-C region of the spectrum is 98 per cent as rapid as in the full light of the source.

An ingenious method of demonstrating the position of the wave lengths responsible for chlorophyll decomposition has also been proposed by DANGEARD. Equal quantities of strong alcoholic extract of the pigment and of alcoholic solution of collodion are mixed and evaporated in a thin layer on a glass plate. This is placed in the spectrum and the places noted where green disappears. Decomposition of chlorophyll occurs in bands corresponding to those of absorption, except that as in photosynthesis the blue-violet seems little active.

Using this same method for mixtures of chlorophyll and other pigments with dyes, the author has studied photosensitization. His conclusions in this field are far from being as novel as he seems to think them, but his method and some of his results are of great interest. Traces of chlorophyll cause rapid decomposition of these dyes in the B-C region, where they do not absorb at all, and traces of bacteriochlorin have the same effect as chlorophyll, except that decomposition is first in the region of its absorption bands in the far and infra red. On the other hand, the carotinoids and bacteriopurpurin have no such photosensitizing effect. The obvious functional correlations are very plausible at least, and are supported by other evidence. Incidentally, the author suggests that the function of the carotinoids is to protect chlorophyll from decomposition in light by absorbing all the adjacent oxygen! Later he admits the possibility of the protecting screen theory of IVANOWSKI.

A further contribution is made to the problem of the wave length used by algae of different color. Blue-green algae and diatoms showed their principal growth bands, both in spectra and behind filters, in the same region as green algae, with an additional band in the far red for the blue-green algae, which have an absorption band at about  $720\text{ }\mu\mu$ . The sulphur bacteria developed only behind filters passing the red and infra red, which is in agreement with the position of their absorption bands. Because of their motility they could be used to form growth bands in the spectrum, but they congregate in a spectrum to form "fixation" bands, as do motile blue-green algae, corresponding to the absorption bands in position.

Less happy are the conclusions regarding the ability of deep water algae to utilize the penetrating yellow-green rays by reason of having chlorophyll with stronger absorption in this region than is exhibited by chlorophyll of land and shallow water plants. The evidence brought forward is very faulty, and in the few cases where strong absorption by bands in the yellow-green is found for chlorophyll from brown and red algae, it is evident that concentration alone is the explanation. There is even reported extraction of a chlorophyll which when separated from the carotinoids had no absorption in the blue-violet, and TSWERR is erroneously cited as having found that his chlorophyllin *a* had very little absorption there. The absorption bands for phycoerythrin are not in agreement with those found by others. Altogether, this chapter of the work is about the least satisfactory.

The author's study of chlorophyll development in the nearly normal spectrum shows a sharp boundary at  $680\text{--}690\text{ }\mu\mu$ , whereas greening is found in varying degrees down to  $440\text{ }\mu\mu$ , with blanched leaves of endive. The maximum development is in the red between B and C, with a weak secondary in the blue-violet. Really more critical is the study using seedlings of *Lepidium* germinated in close ranks in the spectrum. Here the red limit and the maximum are the same as for the leaf, but the lower limit is somewhere near  $570\text{ }\mu\mu$ . Spectroscopic analysis of the extract from seedlings, however, showed traces of green from the seedlings in wave lengths below this, and the limit is not really determined absolutely.

SAYRE<sup>4</sup> recently studied this question, using ray filters, and reports the same red limit, but no limit on the blue side down as far as  $300\text{ }\mu\mu$ , if enough energy is supplied. Actually he does not prove that wave lengths shorter than  $400\text{ }\mu\mu$  are effective, and in the only case in which waves up to  $500\text{ }\mu\mu$  were not transmitted, only traces of green were recorded. The question of the ultra limit of chlorophyll formation is still open. Of great interest is the purely accidental correlative finding of the author that the lower limit of visible chlorophyll development, about  $510\text{ }\mu\mu$ , is the upper limit of phototropic response, which continues strong through the more refrangible part of the spectrum to its limit, here  $320\text{ }\mu\mu$ .

<sup>4</sup> SAYRE, J. D., The development of chlorophyll in seedlings in different ranges of wave lengths of light. *Plant Physiol.* 3:71-78. 1928.

The reviewer has only called attention to some of the more notable features of this very interesting and long neglected work. He believes that the author is correct in the attitude expressed in the preface, that the new methods suggested are more valuable than the data presented. The work is marred by such misprints as invariably 0.02 cm. and 0.001 mm. where obviously 2 cm. and 1 mm. are meant. And the author cannot realize that quantitative measurements of chlorophyll absorption have shown that the absorption bands are only places of local maximum absorption, with the region between bands absorbing only less strongly than the weakest band. Nowhere, also, is any measurement made of light intensity, nor is it intimated that the author realizes that the various ray filters have quite different transmission intensities. Occasionally rather definite conclusions are drawn which are invalid until intensity differences are studied and eliminated. On the other hand, the author has made the bubble-counting method productive of very significant results, and has added several new schemes to those already available for its use. Students of photosynthesis can no longer afford to ignore DANGEARD's work.—H. S. WOLFE.

**Maine vegetation.**—Mount Desert, a rugged and rocky island off the coast of Maine, appears to offer many problems of peculiar ecologic interest. Situated on the tension line between the conifer forests of the north and the deciduous forests of New England, there are within its 100 square miles a variety of forest types ranging from those of northern Canada to those of New Jersey. Among the well developed forest associations are those characterized respectively by spruce, fir, northern hardwoods, white pine, white cedar (*Thuja*), mixed conifers, and pitch pine (*Pinus rigida*).

The environmental factors which control this mosaic of vegetation have recently attracted the attention of MOORE and TAYLOR,<sup>5</sup> who have studied the topography, soil, and climate, as well as the vegetation. Among their data those regarding evaporation are most extensive and most interesting. Pairs of stations established both within and without four of the principal forest types, and maintained throughout the growing seasons of 1921, 1922, and 1923, exhibited remarkable agreement. They plainly demonstrated that not only is the spruce forest the most mesophytic and the *Pinus rigida* association the most xerophytic, but the character of the open sites varies with that of the forest interiors. In other words, the most northern forest type has taken possession of the most mesophytic situations, and the most southern type has persisted in the most arid.

Throughout all the habitats the rates of evaporation are surprisingly high. This is notably shown in the *Pinus rigida* type, where the evaporation is higher than within the same forest much farther south on Long Island. In addition, the average rate in the most mesophytic forest seems to be nearly double that

<sup>5</sup> MOORE, BARRINGTON, and TAYLOR, NORMAN, Vegetation of Mount Desert Island, Maine, and its environment. Brooklyn Bot. Gard. Memoirs 3:1-151. figs. 26. Map. 1927.

found by the reviewer in somewhat similar forests in the Chicago region.<sup>6</sup> These high rates seem to be due to the prevalence of dry southwest winds.

The soil temperatures, taken throughout the three seasons at 6 and 18 inches below the surface, show little difference between the various associations. The *Pinus rigida* has slightly the warmest soil, probably due to its drier character quite as much as to its exposure. It would have been very interesting to have had the range of soil moisture for the three seasons. Without such data the soil analyses, with wilting coefficients and water-holding capacity, have relatively little significance.

In addition to data on environment, the various forest associations are carefully described and their successional relations investigated. Two climax forests are distinguished, the one dominated by spruce (*Picea canadensis* and *P. rubra*), and the other by a mixture of spruce and northern hardwoods, among which sugar maple (*Acer saccharum*) is conspicuous. In the study of the successional relations, the establishment of the tree species does not seem to have received the attention devoted to the herbaceous and shrubby forms.

The study as a whole is to be highly commended for its general excellence, its organization, and its elucidation by photographs, diagrams, and graphs.—G. D. FULLER.

**Regulation of substratum acidity by species of *Sphagnum*.**—It has been the prevalent opinion that *Sphagnum* cannot tolerate lime. STELMACH<sup>7</sup> undertook to investigate the two species, *S. recurvum* and *S. cymbifolium*, by culture methods with regard to the effect (1) of the Ca ion on the development of the moss plants, (2) of the alkaline nature of the substratum on development, and (3) of the moss plants in neutralizing alkaline soil and acidifying acid soil. By using Knopp's calcareous nutrient and calcium-free nutrient solutions, it was found that calcium has no toxic effect in the case of either of the two species tested. While concentrations of 0.2 gm. of  $\text{Na}_2\text{CO}_3$  and  $\text{CaCO}_3$  respectively per liter of solution caused death of all cultures within four weeks, plants of both species thrived in a solution containing 0.125 gm. of  $\text{CaCO}_3$  per liter. It was found that the solutions, after three weeks, gave a neutral test with litmus and not an alkaline test as at the beginning. The neutralization was supposed to be due to the effect of the liberated "wooser" acids. The increase of H-ions hastened development only within certain limits. *S. recurvum* lived within the limits pH 6.8–5.2, with 5.8 as the optimum, while *S. cymbifolium* lived within the limits pH 6.0–4.8, with an optimum of pH 5.4. Both species acidified the weakly alkaline solution and made it fit for their own development. *S. recurvum* defended itself against overacidity by weakening it; *S. cymbifolium* increased the acidity of the acid substratum and died on account of excess.—J. ISENBARGER.

<sup>6</sup> BOT. GAZ. 58:217. 1914.

<sup>7</sup> STELMACH, —, Die Regulation der Substratacidität durch zwei Torfmoose *Sphagnum recurvum* und *S. cymbifolium*). Extrait du Bulletin l'Académie Polonaise et des Lettres Classe des Sciences Mathématiques et Naturelles. Nat. Ser. B. 1926.

# THE BOTANICAL GAZETTE

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## CONTROLLING INFLUENCES IN CORN ROT PROBLEMS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 384

JOSEPH C. IRELAND

(WITH THREE FIGURES)

### Introduction

A statement issued by the Office of Mycology and Disease Survey of the Bureau of Plant Industry (11) indicates that the more important corn-producing states estimated a loss of about 10 per cent of the corn yield for 1926 as a result of root, stalk, and ear rots. HOLBERT and his co-workers (5) suggest a greater loss for the state of Illinois. Crop reports have indicated that the annual loss from corn rots amounts to several million dollars each year.

Plant pathologists have not generally agreed as to a definite limitation of corn rot diseases. The tendency has been to associate some fungus with each pathogenic condition. Ear rots are supposed to be caused by *Diplodia zeae* (Schw.) Lev., stalk rots by *Fusarium moniliforme* Sheldon, root rots and seedling blights by *Gibberella saubinetii* (Mont.) Sacc., scutellum rots by *Rhizopus* spp., and black bundles by *Cephalosporium acremonium*; also some bacteria have been suspected of causing rots. The fungi have been found by various investigators in all parts of the corn plant.

All seem to agree, however, that corn suffers from certain diseased conditions associated with symptoms of a definite nature. Rotting of the roots, weakening and discolorations of the nodes, seed-

ling blight, red streaking of leaves or paleness, weakness of the ear shank, barren stalks, and chaffy kernels, have all been designated as indications of greatest importance. In the field, a very irregular stand and "pale spots," varying in size from a few square rods to several acres in extent, are common. These spots are readily recognized in the older fields of the Corn Belt, east of the Mississippi River. The stalks may range from 2 to 4 feet in height, with a pale color and a tendency to lodge; while the corn about these places will be much taller and of a rich green color. An examination of the individual stalks in these spots will reveal the symptoms commonly known as root rot.

The fact that these spots are surrounded by apparently vigorous corn, often producing high yields, causes one to wonder whether the fungi are the cause of such well defined spots, or whether there is not some ecological or physiological factor controlling the extent of the disease. If it were limited entirely to pathogenic organisms, it appears that the disease would be distributed over the entire field. The problem here undertaken has been to find causes for these pale spots, and, if possible, to suggest a means of eliminating them.

### Historical data

In 1889 BURRILL (2), describing a bacterial disease of corn, mentioned several symptoms commonly attributed to corn rots. He found the roots badly decayed in many cases, the stalks discolored at the lower nodes, streaking of the leaf sheath, and a decay of ears. A "close, very white, feltlike fungus" was mentioned as subsequently infecting the ears. BURRILL states that the disease was first called to his attention in 1882.

Due to poisoning of farm animals by a "cornstalk disease" in Nebraska, interest was revived in the study of corn infection by "molds." In 1903, SHELDON (10) described *Fusarium moniliforme* as an organism producing a pink mold of corn. A similar motive prompted a study of *Diplodia zeae* by VAN DER BIJL (13) in South Africa, in 1916.

During the past few years, more intensive studies have been made by investigators in the agricultural experiment stations of the Corn Belt. One group believes that whatever rot is present is due



to a parasitic fungus; a second group holds the more progressive viewpoint that susceptibility is based upon genetic weaknesses of the corn plant; and a third group supports the idea that malnutrition and a lack of physiological balance are important factors in causing the corn rots.

HOFFER and his co-workers (3) have perhaps advanced the last view more vigorously than any others. They claim that excessive accumulations of iron in the nodes are accompanied by a lack of potassium obtained from the soil, and that a deficiency of nitrogen in the stalk follows a decrease of potassium in the soil. These weakened stalks are said to be increasingly susceptible to disease (4). Microchemical tests applied at the nodes of cornstalks have been recommended as soil indicators for deficiencies of potash and nitrogen. WELTON and others (14) showed that such indicators are unreliable in Ohio. They found the tests decidedly variable, and advised the farmers of that state against the use of such tests for selecting fertilizers, because they are "frequently conflicting and often misleading and unreliable."

HOLBERT and his associates (5) have prepared an exhaustive report of their investigations of the resistance of various strains of corn to fungous infection. They show that it is possible to select a type of corn which is decidedly resistant to infection. The hard, smooth type, with "proper indentation" is shown to be more desirable than the rough starchy type. Their conclusions suggest that earlier plantings are more susceptible to scutellum rot than later ones, and that seedling blights are more common in cooler soils. Selection and crop rotations are indicated as their final means of controlling disease.

VALLEAU (12) has recently shown that a soil-borne fungus, described as a *Pythium*-like organism, is the source of corn root rots. *Gibberella*, *Fusarium*, and *Diplodia* species are secondary, perhaps causing a seedling blight, according to his account. Where continuous cropping in corn is practiced, the infection is said to be greater.

### Experimental methods

Several experiments in greenhouses and in the field were conducted to determine the influences of soils and chemicals upon diseased and disease-free corn. These were begun at the University of

Chicago in 1922. Later they were extended to fields in Indiana and in Oklahoma. The five experiments outlined summarize the work done from 1922 to 1927 inclusive.

#### INFLUENCES OF IRON AND ALUMINIUM

Control cultures of corn were grown to determine the influences of iron and aluminium upon the composition of the corn plant, in the presence and in the absence of a fungus. For this experiment, *Diplodia zeae* was used as a fungus causing typical corn rots. It was most readily isolated from the diseased corn obtained.

An attempt was made to secure corn that was known to be free from pathogenic infection. The varieties Dakota Yellow Dent, Madison Yellow Dent, Longfellow (flint), Reid's Yellow Dent (northern grown), and a strain of 90-Day Corn were obtained from a Chicago seed house. Germination tests showed very slight infection. Samples of seeds from each variety were soaked in distilled water for 12 hours, covered for 3 minutes with a 10 per cent solution of silver nitrate, washed with a N/10 solution of NaOH, and finally washed with sterile distilled water. These grains were then dropped into deep germinating dishes containing sterile dextrose agar. Due to the severe sterilization, only about 10 per cent of the kernels germinated. Since fungi and bacteria did not develop upon the seedlings nor upon the nutrient agar, it may be assumed that the corn used in subsequent studies was reasonably free from pathogenes at the time of transplanting.

Sand pots were used for cultural studies. Eight-inch earthenware pots were coated with paraffin and filled with clean white sand which had been treated for 24 hours with sulphuric acid. The pots of sand were then washed with running water for 12 hours and sterilized in a steam bath for 4 hours.

Stock solutions of nutrient salts were made up separately. Molecular solutions of magnesium sulphate, calcium nitrate, and potassium dihydrogen phosphate were made in sufficient quantities for the entire experiment. Baker "analyzed" chemicals and distilled water were used. Solution type I,  $R_3S_2$ , diluted three and one-half times as recommended by the Committee of the National Research

Council on Salt Requirements of Representative Agricultural Plants, was made for keeping the sand moist and for the nutrition of the corn. The amounts of the salt solution varied from time to time, but an effort was made to apply the same amount to all pots.

When the hypocotyls had reached a length of 0.5 inch, the seedlings were transplanted from the germinating dishes to the sand pots. Each variety of corn was represented by 5 pots: one pot of each variety was set aside as a control series; a second series was inoculated at the cotyledonary node; a third series was treated with a 0.0005 M. solution of aluminium sulphate; a fourth series with a 0.0005 M. solution of aluminium chloride; and a fifth series with a 0.0005 M. solution of ferrous sulphate. The iron and aluminium salts were added to their respective series three times each week for 6 weeks.

After the corn had matured the plants were removed from the pots, the sand washed from the roots, and the total green weights obtained. The plants were then air-dried. A quantitative estimation of the amounts of aluminium and iron was made of the roots, nodes, and internodes of each plant, according to the Official Methods (8). The 3 lower nodes of each stalk were used in each case.

#### INFLUENCE OF FUNGI UPON YIELD

One corn plot was used for 3 years in comparing the yields of diseased and good seed. Beginning in 1922, diseased corn was planted upon 5 acres and good corn was planted upon the adjoining 5 acres, and the experiment was repeated upon the same field during 1923 and 1924. The grain was drilled in rows 3.5 feet apart at the rate of one grain to each foot. The field was a very rich river bottom in southern Indiana. Two hundred and fifty pounds of 2-12-6 fertilizer was added annually to each acre. Perhaps the fertility and soil conditions were as good as might be obtained for corn production.

Seeds for the experiment were obtained by testing with a "modified-rag-doll." Ears that showed no signs of fungous infection during germination were considered good seed; those which showed infection by various fungi, but which were completely viable, were kept for planting the diseased plot. The same procedure was followed

each year. Yields were estimated by weighing each load of corn upon a farm scale as it was husked, in November. Conditions of tillage were the same.

#### INFLUENCES OF TEMPERATURE AND FERTILIZERS

During the 1925 season, an experiment was conducted to determine the influences of temperature and climate upon corn rots. Rich sand-loam was available in northern Indiana and in southern Oklahoma. From soil surveys and from general appearances, these soils were almost identical in composition, the differences being in temperature and moisture. New soil thermographs, regulated identically

TABLE I  
PLANTING PLAN OF FIELD PLOTS

ROW APPLICATIONS	INDIANA CORN	OKLAHOMA CORN	SHOW CORN
1. Potash .....	50 gm. per hill	50 gm. per hill	50 gm. per hill
2. Chilean nitrate .....	25 gm. per hill	25 gm. per hill	25 gm. per hill
3. Cottonseed meal .....	1 lb. per hill	1 lb. per hill	1 lb. per hill
4. Ferrous ammonium sulphate .....	10 gm. per hill	10 gm. per hill	10 gm. per hill
5. Control .....	.....	.....	.....
6, 7. <i>Gibberella saubinetii</i> .....	Sprayed suspension	Sprayed suspension	.....
8, 9. <i>Diplodia zeae</i> .....	Sprayed suspension	Sprayed suspension	.....
10. Control .....	.....	.....	.....

by JULIEN P. FRIEZ of Baltimore, were used to record the temperatures in the fields. The Oklahoma field had grown three crops of cotton but had never been planted to corn since the original prairie sod was broken. The Indiana corn followed a heavy clover crop, where the corn-wheat-clover rotation had been practiced for 50 or 60 years. Both fields were plowed at the same depth and received the same amount of cultivation. Twenty-five rows of corn were planted in each plot, by the ear-to-row method. The first 5 ears in each plot were from a sample of Indiana corn which had placed third in the International Grain and Hay Show, at Chicago. They were of the Reid Yellow Dent variety, and represented the best type of show corn available. A germination test revealed no sign of fungous infection in any of these 10 ears, and the germination was perfect. Twenty ears of Reid's Yellow Dent, free from signs of fungous infection, were selected from seed that had been naturalized to the Red River Valley of Oklahoma. Another 20 ears of corn were selected

from that grown in northern Indiana. Thus each plot contained 10 rows of Indiana corn, 10 of Oklahoma corn, and 5 rows of "show" corn. Each row was 40 rods in length. Estimations of yields were made upon a basis of 20 rows making an acre. Estimations of yield were made in Oklahoma on July 24; those in Indiana on October 5. Table I indicates the applications of chemicals and fungi which were made upon these duplicate plots. They were made when the two lots of corn were at the same stages of development. The Oklahoma season was more than one month in advance of the Indiana season.

#### INFLUENCES OF MOISTURE AND SOIL TEXTURE

An experiment was conducted to determine the influences of moisture and soil texture upon the composition of the corn plant and upon its susceptibility to disease. Forty 5-gallon tin oilcans were used; 20 were filled with a heavy yellow clay, and 20 with rich garden soil. Ten of each were planted with a disease-resistant strain of corn, and the remaining cans were planted with susceptible corn. The seed was kindly provided by Dr. G. N. HOFFER of Purdue University, and both strains had been inbred for several generations. The grains were surface-sterilized and germinated upon agar plates to make sure that no fungi were present when the experiment started. Ten of the clay cans and 10 of the loam cans were watered daily to maintain an excess of moisture; the others were watered only enough to keep the corn growing.

After the corn had been planted three weeks, 5 gm. of ferrous ammonium sulphate, dissolved in one liter of distilled water, was added to one can in each of the four groups. The treatment was repeated weekly for 5 weeks. Aluminium sulphate was added in the same amounts to four other pots at the same time. Applications of fungous cultures were made to cans in each group by spraying the corn with water suspensions. When the first coleoptiles began to appear above the ground spraying was begun. A second application was made when the stalks were 6 inches high.

The corn was harvested at the time of tasseling, and weights and analyses were recorded. Quantitative estimations of reducing sugars, total nitrogen, ferric and aluminium oxides, and phosphorus were made, according to the Official Methods (8).

## INFLUENCES OF COLLOIDAL SOIL

An experiment was begun in the greenhouses of the University of Chicago to determine the influences of a colloidal silt loam upon the development of roots, and upon the susceptibility of corn to infection from fungi. The work was suggested by observations made during several trips across the Corn Belt. Practically all the "pale spots" in corn fields are found in places where the clay is inclined to

TABLE II  
PLANTING PLAN FOR CORN GROWN IN CANS

CAN NO.	WET CLAY	DRY CLAY	WET LOAM	DRY LOAM
1 .....	Ferrous ammonium sulphate	Ferrous ammonium sulphate	Ferrous ammonium sulphate	Ferrous ammonium sulphate
2 .....	Aluminium sulphate	Aluminium sulphate	Aluminium sulphate	Aluminium sulphate
3 .....	Diplodia zeae	Diplodia zeae	Diplodia zeae	Diplodia zeae
4 .....	Gibberella saubinetii	Gibberella saubinetii	Gibberella saubinetii	Gibberella saubinetii
5 .....	Control	Control	Control	Control

TABLE III  
ARRANGEMENT OF SOILS AND TREATMENT

SOIL TREATMENT	FUNK'S DISEASE-FREE SEED		INFECTED SEED	
	Silt loam (gm.)	Greenhouse soil (gm.)	Silt loam (gm.)	Greenhouse soil (gm.)
Ferrous ammonium sulphate....	10	10	10	10
Lime.....	25	25	25	25
Manure.....	200	200	200	200
Sodium nitrate.....	10	10	10	10
Potassium chloride.....	10	10	10	10
Control.....				

"puddle," or where the drainage is poor, producing what the farmers call "cold places." Clermont silt loam was obtained from a field which indicated these conditions. The lime requirement for general agricultural purposes was determined by the Soiltex indicator. Lamotte and potentiometer estimations were made of the pH concentration. The percentage of colloidal material in the soil was estimated by the use of the most recent hydrometer method of BOUYOUCOS (1).

Twelve glazed pots containing silt loam, and 12 containing the best greenhouse soil were steam-sterilized for 8 hours. Surface-sterilized grains of Funk Brothers' "inbred, high-oil, disease-free, utility type" of corn were germinated upon sterile agar in Petri dishes and planted in 6 pots of each kind of soil. A second planting was made with corn from the same source which was known or was thought

TABLE IV A

TREATMENT	TOTAL GREEN WEIGHT	TOTAL DRY WEIGHT	TOTAL ASH PER CENT DRY WEIGHT	Al <sub>2</sub> O <sub>3</sub> TOTAL DRY WEIGHT		Fe <sub>2</sub> O <sub>3</sub> TOTAL DRY WEIGHT	
				Nodes	Roots	Nodes	Roots
Control..... Infected.....	Longfellow (flint)						
	387 278	132 63	2.1 3.4	0.029 0.019	0.014 0.011	0.009 0.008	0.015 0.0094
	90-Day						
	252 19	113 6	2.02 3.21	0.007 0.004	0.0074 0.0052	0.019 0.010	0.018 0.018
Control..... Infected.....	Reid's Yellow Dent (northern)						
	252 107	113 27	2.21 2.71	0.0037 0.0031	0.0058 0.0056	0.008 0.005	0.017 0.013
	Madison Yellow Dent						
	387 178	132 53	2.1 3.4	0.0036 0.0025	0.0148 0.0112	0.004 0.003	0.017 0.014
Control..... Infected.....	Dakota Yellow Dent						
	222 96	94 27	2.07 3.5	0.0038 0.002	0.0132 0.0067	0.004 0.003	0.013 0.009

to be infected with *Gibberella saubinetii*. The grains were surface-sterilized and germinated upon sterile agar. Those grains which were viable but which showed signs of *Gibberella* infection were planted in the remaining clay and greenhouse soil pots. Treatments of the respective groups are shown in table III.

Five kg. of soil was used in each pot. With the exception of the manure, which was applied dry, the chemicals were applied by dissolving the amounts indicated in one liter of water and applying at the rate of 100 cc. daily until the entire amounts had been added.

A soil temperature of  $21^{\circ}$  was maintained, and water was added daily. The chemist of the Ohio Agricultural Experiment Station, Wooster, Ohio, estimated that the silt loam contained the following nutrients: nitrogen 0.154 per cent, phosphorus 0.40 per cent, potassium 2.07 per cent.

The root systems were washed out carefully, and the dry weights of the entire plants were recorded at the end of the experiment.

TABLE IV B

TOTAL GREEN WEIGHT	TOTAL DRY WEIGHT	TOTAL PER CENT ASH	Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>				Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>				Al <sub>2</sub> Cl <sub>6</sub>			
			Al <sub>2</sub> O <sub>3</sub>		Fe <sub>2</sub> O <sub>3</sub>		Al <sub>2</sub> O <sub>3</sub>		Fe <sub>2</sub> O <sub>3</sub>		Al <sub>2</sub> O <sub>3</sub>		Fe <sub>2</sub> O <sub>3</sub>	
			Node	Root	Node	Root	Node	Root	Node	Root	Node	Root	Node	Root
Longfellow														
405	73	2.1	0.218	4.9	0.129	0.34	.....	.....	.....	.....	.....	.....	.....	.....
371	53	2.38	.....	.....	.....	.....	0.236	0.23	0.140	0.48	.....	.....	.....	.....
486	69	2.54	.....	.....	.....	.....	.....	.....	.....	.....	0.291	1.78	0.084	0.24
90-Day														
377	145	1.7	0.56	4.04	0.114	0.228	.....	.....	.....	.....	.....	.....	.....	.....
287	94	2.5	.....	.....	.....	.....	0.190	0.243	0.23	0.83	.....	.....	.....	.....
436	109	3.1	.....	.....	.....	.....	.....	.....	.....	.....	0.19	0.62	0.140	0.237
Reid's Yellow Dent (northern)														
282	101	2.5	0.402	1.17	0.271	0.19	.....	.....	.....	.....	.....	.....	.....	.....
293	74	2.1	.....	.....	.....	.....	0.24	0.34	0.291	0.211	.....	.....	.....	.....
332	121	3.7	.....	.....	.....	.....	.....	.....	.....	.....	0.38	1.14	0.22	0.204
Madison Yellow Dent														
356	81	1.8	0.329	0.31	0.26	0.24	.....	.....	.....	.....	.....	.....	.....	.....
406	140	2.3	.....	.....	.....	.....	0.29	0.169	0.306	0.258	.....	.....	.....	.....
391	128	2.5	.....	.....	.....	.....	.....	.....	.....	.....	0.34	0.205	0.29	0.205
Dakota Yellow Dent														
137	47	1.99	0.236	0.74	0.246	0.21	.....	.....	.....	.....	.....	.....	.....	.....
231	68	2.4	.....	.....	.....	.....	0.216	0.182	0.214	0.331	.....	.....	.....	.....
232	73	3.08	.....	.....	.....	.....	.....	.....	.....	.....	0.34	1.93	0.207	0.182

### Experimental data

#### INFLUENCES OF IRON AND ALUMINIUM

The corn planted in the sand pots grew rapidly. Stalks from each variety, inoculated with *Diplodia zeae*, began to show signs of infection within a week. The characteristic red and brown streaks were



evident. No marked difference was noted in the susceptibility of the five varieties. Pure cultures of the fungus were obtained from the inoculated stalks at various intervals during the experiment.

Stalks treated with aluminium chloride, aluminium sulphate, and ferrous sulphate did not show symptoms of the root and stalk rots, but the growth was rather more vigorous than the controls. Quantitative estimations of the first three nodes and of the roots were made of all stalks for the amounts of ferric and aluminium oxides present. These are summarized in tables III and IV. The tabulations also show the green and dry weights, as well as the ash content.

#### INFLUENCE OF FUNGI UPON YIELDS

Table V summarizes the yields of disease-free and of diseased corn upon river bottom land during a period of three years. As previously stated, conditions were very favorable for growing corn during the entire period.

TABLE V  
SUMMARY OF YIELDS FOR 3 YEARS

YEAR	FIVE ACRES GOOD SEED (BUSHELS)	FIVE ACRES DISEASED SEED (BUSHELS)
1922.....	401	405
1923.....	389	406
1924.....	360	452
3-year total.....	1150	1263

Yields were estimated by weight of corn taken from the field. A bushel was considered 72 lb.

#### INFLUENCES OF TEMPERATURE AND FERTILIZERS

The soil temperatures of Oklahoma and Indiana cannot be compared from the point of view of corn growth. During the summer of 1925 Indiana had almost an ideal corn season, while Oklahoma did not. No rain fell in the latter state after June 15 until the end of the experiment. The scorching sun and hot winds reduced the yield almost to nothing. It is interesting to note that early varieties of corn, planted in March, matured sufficiently early to avoid the hot weather and produced a fair crop, while the later varieties almost perished.

The yields given in table VIII were estimated by weighing the ears produced upon 40 rods. Twenty such rows are considered an acre; 72 lb. is considered a bushel.

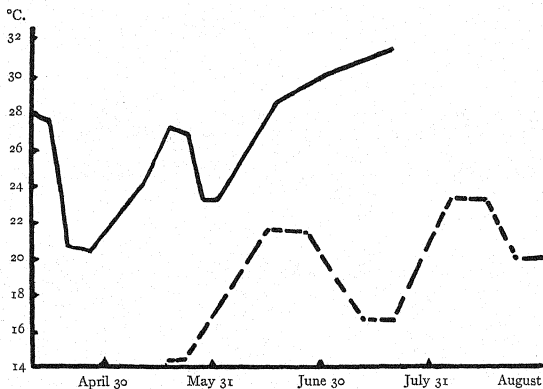


FIG. 1.—Graphic comparison of Oklahoma and Indiana soil temperatures: vertical column represents range of temperatures (°C.); horizontal column indicates time; solid line indicates Oklahoma temperatures; dotted line represents Indiana temperatures.

TABLE VI

YIELDS OF INDIANA REID'S YELLOW DENT IN OKLAHOMA AND IN INDIANA

Row No.	Treatment	INDIANA YIELD PER ACRE (bushels)	OKLAHOMA YIELD PER ACRE (bushels)
1.....	Potash	71	5
2.....	Chilean nitrate	69	3.5
3.....	Cottonseed meal	67	4
4.....	$\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot \text{H}_2\text{O}$	70	3
5.....	Control	73	6
6, 7.....	Gibberella saubinetii	71 and 69	7 and 8
8, 9.....	Diplodia zeae	64 and 68	5 and 3.5
10.....	Control	72	4
Average yield.....		69.4	4.9

## INFLUENCES OF MOISTURE AND SOIL TEXTURE

The following data were obtained by weighing the entire corn plants after washing and drying to a constant weight. These plants were grown in the 5-gallon cans of soil.

TABLE VII

YIELDS OF OKLAHOMA REID'S YELLOW DENT IN OKLAHOMA AND IN INDIANA

ROW NO.	TREATMENT	INDIANA YIELD PER ACRE (BUSHELS)	OKLAHOMA YIELD PER ACRE (BUSHELS)
1.....	Potash	43	6
2.....	Chilean nitrate	47	7
3.....	Cottonseed meal	40	3
4.....	$\text{FeSO}_4 \cdot (\text{NH}_4)_2\text{SO}_4 \cdot \text{H}_2\text{O}$	38	2
5.....	Control	45	9
6, 7.....	Gibberella saubinetii	46 and 42	4 and 5
8, 9.....	Diplodia zeae	39 and 44	7 and 3
10.....	Control	46	6
Average yield.....		43	5.2

TABLE VIII

YIELDS OF SHOW CORN IN INDIANA AND OKLAHOMA

ROW NO.	TREATMENT	INDIANA YIELD PER ACRE (BUSHELS)	OKLAHOMA YIELD PER ACRE (BUSHELS)
1.....	Potash	62	12
2.....	Chilean nitrate	63	2
3.....	Cottonseed meal	58	7
4.....	$\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot \text{H}_2\text{O}$	64	9
5.....	Control	61	11
Average yield.....		61.6	8.2

TABLE IX

COMPARATIVE WEIGHTS OF CORN PLANTS GROWN FROM RESISTANT SEED

CAN NO.	TREATMENT	CLAY		GARDEN SOIL	
		Wet	Dry	Wet	Dry
		Gm. dry weight	Gm. dry weight	Gm. dry weight	Gm. dry weight
1.....	$\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot \text{H}_2\text{O}$	246	102	420	163
2.....	$\text{Al}_2(\text{SO}_4)_3$	257	75	450	139
3.....	Diplodia zeae	191	69	301	168
4.....	Gibberella saubinetii	97	104	275	155
5.....	Control	262	109	450	313

In the preceding tables the terms wet and dry refer to conditions of the soil; dryness means about 40 per cent of the water-holding capacity of the soil; wet, 60 per cent.

Analyses were made of the plants indicated, estimating quantitatively the constituents listed in table XI. For iron and aluminium

TABLE X  
COMPARATIVE WEIGHTS OF CORN PLANTS GROWN FROM SUSCEPTIBLE SEED

CAN NO.	TREATMENT	CLAY		GARDEN SOIL	
		Wet	Dry	Wet	Dry
		Gm. dry weight	Gm. dry weight	Gm. dry weight	Gm. dry weight
1.....	$\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot \text{H}_2\text{O}$	229	107	390	104
2.....	$\text{Al}_2(\text{SO}_4)_3$	241	91	405	113
3.....	<i>Diplodia zeae</i>	173	62	349	88
4.....	<i>Gibberella saubinetii</i>	96	56	192	52
5.....	Control	194	86	381	120

TABLE XI  
CHEMICAL COMPOSITION OF RESISTANT CORN

CONSTITUENTS	CLAY		GARDEN SOIL	
	Wet	Dry	Wet	Dry
	Per cent dry weight	Per cent dry weight	Per cent dry weight	Per cent dry weight
Reducing sugar.....	1.76	1.95	1.85	1.31
Phosphorus.....	0.123	0.096	0.23	0.14
Nitrogen.....	0.79	0.62	0.89	0.48
Ferric oxide.....	0.05	0.047	0.07	0.058
Aluminium oxide.....	0.135	0.21	0.097	0.106

determinations the three nodes just above the ground were taken. These were dried to a constant weight, ashed, and the estimations made from the ash. The remainder of the plant was ground as finely as possible with a food chopper and preserved in alcohol, made up to 80 per cent.

#### INFLUENCES OF COLLOIDAL SOIL

Clermont silt loam and the greenhouse soil were tested to determine the limestone requirement for general agricultural purposes and for the pH values. Table XIII also indicates the percentage of

colloids present in the respective soils, as determined by the Bouyoucos hydrometer method (1).

The corn grown in the 24 pots of this experiment (table XIII) was harvested early, in order to make determinations for a more

TABLE XII  
CHEMICAL COMPOSITION OF SUSCEPTIBLE CORN

CONSTITUENTS	CLAY		GARDEN SOIL	
	Wet	Dry	Wet	Dry
	Per cent dry weight	Per cent dry weight	Per cent dry weight	Per cent dry weight
Reducing sugar.....	2.11	2.22	1.86	2.08
Phosphorus.....	0.151	0.091	0.113	0.083
Nitrogen.....	0.625	0.341	1.102	0.89
Ferric oxide.....	0.029	0.080	0.075	0.064
Aluminium oxide.....	0.181	0.310	0.121	0.154

TABLE XIII  
ACIDITY AND COLLOIDAL PERCENTAGE IN SOILS

METHOD USED	SILT LOAM	GREENHOUSE SOIL
Soiltex.....	3 tons limestone	Neutral
LaMotte comparator.....	pH value 5.8	pH value 6.6
Potentiometer.....	pH value 4.5	pH value 6.71
Hydrometer colloid content.....	25.3 per cent	17.5 per cent

TABLE XIV  
YIELDS ON SILT LOAM AND GREENHOUSE SOILS EXPRESSED  
IN GM. OF DRY WEIGHT OF CORN

SOIL TREATMENT	FUNK'S DISEASE-FREE SEED		INFECTED SEED	
	Silt loam	Greenhouse	Silt loam	Greenhouse
Ferrous ammonium sulphate....	22.4	54.7	33.8	45.2
Lime.....	11.5	35.6	18.5	41.3
Manure.....	34.5	59.1	37.0	57.8
Sodium nitrate.....	17.9	51.8	25.6	53.3
Potassium chloride.....	41.7	58.6	34.0	53.4
Control.....	20.4	43.9	18.5	53.0

extensive field experiment. The corn had grown for 6 weeks, and had reached a height of 2 feet when it was removed from the pots and washed. It was air-dried and weighed as shown in table XIV.

### Discussion of results

In the sand cultures, treatment of the growing corn with solutions of aluminium chloride, aluminium, and ferric sulphates did not produce a pathogenic condition similar to the root, stalk, or ear rots of corn. The sulphates of iron and aluminium seemed to improve the growth. A number of quantitative estimations were made of the nodes and roots of diseased and control stalks of corn, to determine the comparative amounts of aluminium and iron compounds present. The former usually contained more of these elements than did the controls.

The 3-year test of yields of diseased and disease-free seed was suggested by an extensive seed corn testing program conducted by the writer during the seasons of 1921 and 1922, in a southern Indiana county. Skeptical farmers questioned the elimination of corn diseases by selection with rag-doll germinators, then in vogue in Indiana. Suggestions were offered that a comprehensive experiment be conducted under the direction of the writer. Tests were made of the seed and the planting was supervised as outlined. The yield is not unusual, considering the fact that some of the best seed and best methods of cultivation were used. MELCHERS and JOHNSTON (7) confirm the results of this experiment. They show that a large percentage of the seed ordinarily used is infected with *Fusarium moniliforme*, and that seedling vigor is more important than the absence of fungi.

Observing the two plots of corn, it was impossible to detect any difference in the vigor of the stalks. The fact that the diseased corn produced a few more bushels during the 3 years was probably due to the fact that the stand was not so heavy. There undoubtedly was some seedling blight, which eliminated a few of the weaker stalks, giving an opportunity for the development of larger ears. The stand of the disease-free corn was thicker, and the ears did not grow so large, because of overcrowding.

It may be concluded that as long as fungous infection does not affect seed viability to a great extent, the yield is not decreased.

While the Oklahoma corn yields were almost a failure, some interesting facts were shown in connection with the limits of temperatures at which corn will produce a crop. Until the tasseling stage,

the Oklahoma field was more promising of definite results than that in Indiana. The applications of Chilean nitrate caused the second rows of each planting to grow almost a foot taller than the others. All other applications had no visible effect upon the growth of stalks. On June 15, an extremely hot, dry wind began to blow, at the time when the ears were forming. Within a few days the entire field had turned to the sickening brown characteristic of Oklahoma landscapes at that time of year. The corn which had been treated with Chilean nitrate developed a very pronounced case of root and stalk rot during the process. The nodes developed discolorations, the stalks broke of their own weight, and the roots decayed. Frequent attempts to isolate *Diplodia* and *Gibberella* from these stalks failed. A bacterium which seems to cause a vegetable rot was present in nearly every case. It might have been similar to that described by ROSEN (9) in Arkansas.

Judging from yields, frequent sprayings of plants with suspensions of spores of the fungi did not produce disease. Large quantities of the fungi had been grown upon cornmeal, in large Erlenmeyer flasks, for the purpose of spraying. Field applications of fungi generally associated with corn rots did not produce symptoms attributed to the diseases, if the plants were growing vigorously. HOLBERT and others (5) have emphasized this fact in their reports.

The influences of soil texture and moisture content were investigated under more controlled conditions. Yellow clay soil did not produce the growth naturally expected from garden soil, and the stalks were apparently much more susceptible to infection from *Diplodia* and *Gibberella*. Stalks were sprayed rather frequently and the usual symptoms developed. Infection was less pronounced in the garden soil. Root rots were evident to a greater extent in the dry cans than in the wet. It is interesting to note that the "resistant" corn was diseased as much as the "susceptible" in this experiment. The fact that both had been inbred in opposite directions was not an appreciable factor. The former had a more nearly normal kernel, and naturally produced a more vigorous stalk, as shown by the controls.

Iron and aluminium sulphates did not have an injurious effect upon corn. In the wet clay the growth was more vigorous than in

the controls. The leaves were a darker green. It is probable that these salts had a tendency to flocculate the clay. In making the analysis of the lower nodes of the stalks, it was found that corn growing in heavy clay soils has a higher aluminium and iron content than corn growing in sandy loams. This does not seem to be associated with susceptibility to diseases directly, but is related rather to assimilation. The phosphorus content was greater in corn grown in garden soil, and the nitrogen content was also higher. HUME (6) states that the ash constituents of corn plants do not correlate with a normal nor an abnormal condition, but differ with individual plants.

From this experiment, it is evident that the physical and chemical conditions of the soil are of greater importance in the development of corn plants than the presence or absence of fungi.

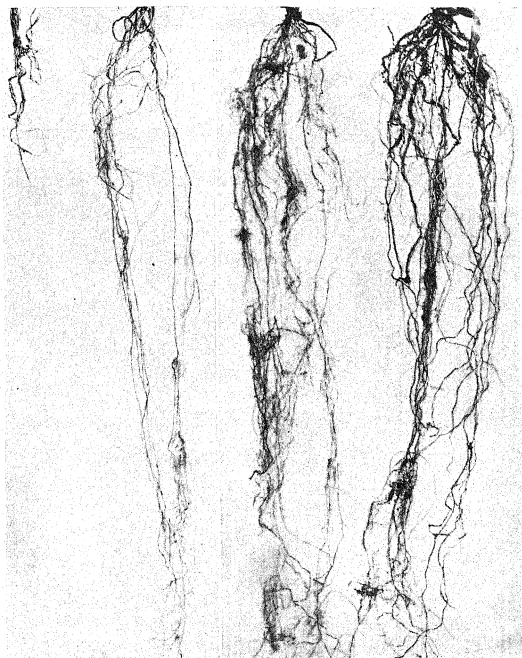
Following the suggestions of soil influences which were noted in this experiment in Oklahoma, the writer had an opportunity to observe the spotted condition of diseased cornfields during several trips through the leading corn-producing states. The "puddled soils" or "gumbo" produced diseased corn, and the "pale spots" are usually limited to these areas.

A silt loam, commonly known as white clay, was obtained to demonstrate the lack of proper aeration in the soil. It showed a limestone requirement of 3 tons per acre. With a LaMotte comparator set, the pH value was estimated at 5.8. A potentiometer reading of a suspension of the same soil indicated a pH value of 4.5. The hydrometer methods of colloid determination indicated that the silt loam had a colloidal content, almost 50 per cent greater than that of greenhouse soil.

Applications of materials were made to the pots of soil, to determine which would provide the best flocculant for field experiments. The aim was to use those correctives which are within the range of agricultural practice. From the dry weights of the plants it is quite evident that potassium chloride and manure are the most successful in producing vegetative growth. While lime corrected the acidity, it did not improve the growth but rather injured the corn. Sodium nitrate might well be expected to prove beneficial, but it did not.



Ferrous ammonium sulphate had been found to be a very effective flocculant of the clay, and it was used for that purpose. Along with



FIGS. 2, 3.—Fig. 2 (left), finely branched roots of a plant grown in colloidal soil; coarser roots grown in greenhouse soil; fig. 3 (right), roots of corn plants inoculated with *Gibberella saubinetii*; badly decayed roots grew in colloidal soil, others grew in greenhouse soil.

potassium, it may have had some nutritive value which would account for the increased production. Comparisons were made by a

duplicate experiment with Funk's best seed corn and with seed from the same source, which was known to be infected with *Gibberella saubinetii*. Under the same conditions the growths were similar.

Washing the soil away from the roots revealed a marked difference in the branching of the root systems. Those grown in silt loam were much branched and finely divided. Since the experiment was conducted with sterilized soil, it is quite probable that the much branched root is more susceptible to rot infections than those coarser roots from the loose garden soil. A detailed study of the methods of infection in corn seedlings shows that lesions occur at points where the secondary roots break through the cortex. With an abnormal development of secondary branches, it is evident that such corn plants will show root rots more readily than those which have the normal branching. Fig. 2 shows this difference in branching. Fig. 3 shows the difference in response to inoculation with a pure culture of *Gibberella saubinetii*. The larger root growing in greenhouse soil shows some infection, but the smaller plant shows almost complete decay.

### Summary

1. Under optimum conditions for corn production, if the seed is viable, fungous infection of the type usually called root rot does not affect the yield.
2. Extremely high soil temperatures do not increase the susceptibility of corn to diseases, although the corn may be killed by the extreme heat. The Oklahoma soil temperature approached the thermal death point of maturing corn.
3. Applications of iron and aluminium salts do not increase the susceptibility of corn to diseases. In the case of clay soils, a limited application seems to be beneficial, perhaps as a flocculant.
4. Colloidal soils induce an extraordinary development of secondary roots in corn plants and render them more susceptible to fungous infection. Lesions originate at points where secondary roots break through the cortex.
5. A large percentage of corn rots may be eliminated by flocculating colloids in the soil, allowing the normal development of the plant.

The writer desires to acknowledge his appreciation of the valuable and timely suggestions of Dr. C. A. SHULL and Dr. S. V. EATON of the Department of Botany of the University of Chicago. Those farmers who have contributed so generously of their land and labor deserve much credit for the results of the experiments.

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## SEED GERMINATION IN CERTAIN NEW MEXICO RANGE GRASSES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 385

CAROLA V. JACKSON

(WITH FIVE FIGURES)

### Introduction

Much work has been carried on in recent years in regard to the viability and germination of seeds of various plants. Seed-testing studies are highly important, because the results obtained influence or affect the work of the farmer, the floriculturist, the amateur gardener, and the rancher or ranger. These people cannot afford to plant seeds, expecting a 95 per cent germination and then perhaps having only a 50 per cent germination, due to poor selection of seed, to its immaturity, or to its adulteration with weed seeds.

Among the plants studied for germination the grasses have a prominent part, and this is especially true of the range grasses of the west. It is necessary for the rancher or ranger to know about what percentage of the seeds of the grasses covering his grazing lands he can expect to germinate, since he can then estimate the amount of grazing his lands will tolerate without becoming depleted. He must know whether his grasses propagate themselves vegetatively or by seed, or by both means.

Various studies have been made with reference to seed germination, and although they have no direct bearing upon the problem which the writer undertook, yet they gave ideas for some tests which were performed after the major problem was completed. The following are short summaries of the work of some of the investigators in the field of seed germination.

In general it is believed that the delay in after-ripening is due to the characters of the embryo and of the seed coat. Miss ECKERSON (6) found that there is a series of metabolic changes going on in the embryo during the period of after-ripening. At first the acidity is increased, and correlated with it is increased activity of catalase

and peroxidase. By treating the embryos with dilute acids such as hydrochloric, butyric, and acetic, the after-ripening period can be reduced very much. Those embryos which are treated increase their water-holding power, acidity, and amount of peroxidase more rapidly; and the oxidase appears sooner than in the untreated embryo.

PAMMEL and KING (14) planted mature and immature seeds in the fall and in the spring. In general, stratification in sand and freezing were favorable to germination. *Asclepias syriaca* showed 12 per cent germination, *Ambrosia psilostachya* 18 per cent, *Chenopodium album* 88 per cent, and *Xanthium canadense* 25 per cent.

PACK (13) discovered that the germination of non-after-ripened *Juniperus* seeds under ordinary conditions is very small, amounting to 1 per cent. These seeds are protected by a semi-permeable and thick coat which makes up 75 per cent by weight of the entire seed. Acids enter very slowly, he found, while bases, silver, and mercury salts enter rapidly. PACK thinks that while the coat may act as a protection against fungal attack, and may prevent water-imbibed seeds from expanding and bursting the tissues before after-ripening is accomplished, it takes little or no part in the dormancy of after-ripening of the seed. He was unable to force the germination of non-after-ripened *Juniperus* seeds by high temperature, alternate temperatures, wounding, warm bath, dry air, removal of coats; or by treatment with hydrogen peroxide, mercuric chloride, ether, carbon dioxide, oxygen, light, soil, dilute acids, dilute bases, nitrates, sulphates, or strong acids. Freezing and thawing as such have no forcing action on germination of the *Juniperus* seeds, neither do they hasten after-ripening. They bring about chemical changes in the seed, but these changes are different from those occurring during after-ripening. When seeds are about ready to germinate, PACK found that they are very sensitive and are killed by exposure to 5° C. The *Juniperus* seed has a dormant embryo that must be after-ripened before germination.

Mrs. DAVIS (4) found the sterilization of the naked seeds of *Cornus florida* difficult, due to the fact that the inner testa is very thin or the endosperm rich in food and the seeds are frequently infested with molds. She concludes that the pericarp, the outer testa, and the inner testa take no part in causing the delay; the moisture

intake with the pericarp and testas intact is as high as in seeds with these removed. After-ripened seeds break the pericarp readily. Delay is not caused by an immature embryo, as it is well differentiated several weeks prior to shedding. After-ripening of the dormant seed is favored by low temperatures, 0-5° C.

*Rubus* seeds vary according to species in the time required to weaken the coat with sulphuric acid. After the carbonized coats have been removed from the seeds they must be carefully sterilized, since treated seeds are very susceptible to molds. The delay in *Sphaeralcea remota* is due to the impermeable cuticle forming the outer layer of the seed coat. After this coat has been subjected to a 2-3 hour treatment with sulphuric acid it becomes permeable. Chipping slightly helps, as this method makes it possible for water to enter the seed. Mrs. DAVIS found that untreated seeds failed to swell or germinate, but that may have been due to the fact that the seeds were gathered late in the season. Treated seeds germinated and grew for a time in distilled water, and all seedlings showed remarkable vigor.

CROCKER (3) claims that in *Xanthium canadense* delayed germination is generally due to the seed coat rather than to the embryo. In the upper cockle-bur seed the delay is due to the exclusion of oxygen by the seed coat. No germination appeared in *Iris* seeds because the cap and endosperm stopped the absorption of water before the needed amount was obtained by the embryo. Those seed coats which exclude water are better for causing delay than those which exclude oxygen, because there is less respiration. The length of delay is due in nature to the persistence of the seed coats. In the case of *Xanthium*, the bur helps in causing the upper seed to germinate later. Seed coats reduce the oxygen supply, especially in the upper seed. High temperature brings about the germination of the upper seeds with the coats intact by raising the respiration ratio, which increases the rate of diffusion of oxygen through the seed coat.

DUVEL (5) has discovered that the factors affecting the vitality of the seed are maturity, weather conditions at time of harvesting, methods of harvesting, and curing. Immature seeds sown soon after gathering usually germinate readily, but if they are stored they soon lose their vitality. Seeds which are harvested in damp rainy weather are much weaker in vitality. By special care the life of the seed once

injured may be prolonged. Since moisture affects the longevity of seeds, they must be kept in a dry place where the temperature is low. DUVEL found that seeds treated in a sulphuric bath or in a vacuum usually showed delayed germination because the seed coat has hardened. In order to keep the vitality of the seed, it is better to have as little respiration as possible. Respiration brings about a chemical activity in the cells, which causes energy to be transformed, resulting eventually in the death of the seed. Respiration in the light is the same as in the dark if moisture and temperature conditions are the same.

FAWCETT (8) worked with 92 samples of weed seeds, representing 52 species. The seeds were collected in September, October, and November. Fifty seeds of each sample were placed in sand in boxes under the benches in the greenhouse. Every month from November to May this was repeated. Samples were also placed in sacks inside of a wooden box and a thin layer of sand placed around them. The boxes were then sunk in the ground so that just the top was exposed. Comparisons were made with the samples kept indoors. In April both lots were planted outdoors. FAWCETT concludes that weed seeds with thick seed coats require a more or less extended period of rest after maturity. Mustard and pepper grass seeds require little time for rest. Drying out weakens the vitality of nearly all weed seeds, and exposure to the natural periods for best seed germination, fall and spring, increases the power of germination.

HIMMEL (11) also believes that low percentage of germination for honey locust is due to the seed coats, for when the seeds had been treated with concentrated sulphuric acid the germination percentage increased very much. The older the wheat seeds the lower the germination percentage, but *Amaranthus* seeds displayed greater germination in the older seeds. The life of a dandelion seed is less than 8 years, but even the old seeds show good catalase activity. *Typha* seeds germinate better if they are pricked. Only very dilute acids and bases have any forcing effect on *Typha* seeds.

Miss EVANS (7) found that in after-ripened seeds with coats untreated, the restricting effect of the coats showed particularly at low temperatures 8-10° and 11.6° C., and again at high temperatures, 42° for Washington seeds and 46.1° for Indiana seeds. In both

cases these effects can be lessened by treating the coats with  $H_2SO_4$ , or abrading them with sand.

ATWOOD (1) found that there was less delay in germination in *Avena fatua* after the shell coats had been removed. Restriction of the oxygen supply by the seed coat acts as a limiting factor in germination. These seeds do not seem to be affected by light during germination. ATWOOD concludes that after-ripening occurs with the drying of the seed, but independent of the water content, as air-dried seeds soon after harvest yield lower germinative percentages than seeds of similar moisture content the following spring. Exclusion of water by the true seed coat does not explain after-ripening according to ATWOOD.

### Material and methods

The primary purpose of this investigation was to discover the percentage of germination of the 1926 seeds of the following grasses:

- |                                   |                                   |
|-----------------------------------|-----------------------------------|
| 1. <i>Hilaria mutica</i>          | 10. <i>Bouteloua gracilis</i>     |
| 2. <i>H. mutica</i>               | 11. <i>Bouteloua eriopoda</i>     |
| 3. <i>Muhlenbergia porteri</i>    | 12. <i>Sporobolus giganteus</i>   |
| 4. <i>Sporobolus airoides</i>     | 13. <i>Aristida longiseta</i>     |
| 5. <i>Aristida purpurea</i>       | 14. <i>Sporobolus flexuosus</i>   |
| 6. <i>Scleropogon brevifolius</i> | 15. <i>Aristida longiseta</i>     |
| 7. <i>Bouteloua eriopoda</i>      | 16. <i>Bouteloua curtipendula</i> |
| 8. <i>Muhlenbergia arenicola</i>  | 17. <i>Sporobolus auriculatus</i> |
| 9. <i>Sporobolus cryptandrus</i>  |                                   |

Studies of these grasses have been made in previous years at the government laboratories in Washington, D.C., but the 1926 seeds were sent to the Hull Laboratory of the University of Chicago for experimentation by the writer. The seeds were collected by R. S. CAMPBELL on the Jornada Range Reserve Station at Las Cruces, New Mexico, and through him the following seeds from 1925 were obtained:

- |                                |                                   |
|--------------------------------|-----------------------------------|
| 20. <i>Sporobolus airoides</i> | 24. <i>Epicampes emerslyei</i>    |
| 21. <i>S. auriculatus</i>      | 25. <i>Muhlenbergia arenicola</i> |
| 22. <i>S. cryptandrus</i>      | 26. <i>Panicum obtusum</i>        |
| 23. <i>S. flexuosus</i>        |                                   |

The seeds were still in the glumes on the spikes, hence it was necessary to separate not only the florets from the spikelets, but also



to remove the palea and lemma from the seeds. The seeds for the tests were selected at random, so that the results would be as representative as possible. Since some of the samples contained relatively few seeds, it was found necessary to use smaller amounts of seeds for some tests. In all cases the seeds were soaked for one half-hour in a 0.25 per cent solution of uspulun and then rinsed (washed) in distilled water twice for 15 minute periods. The blotting paper was also treated with the uspulun solution, and the Petri dishes containing cotton and filter paper were sterilized by autoclaving. These precautions were taken to insure the seeds against black mold attack. Only in a few instances were the seeds attacked by molds.

All seeds were tested at 25° C. in the moist chamber germinating oven in blotters and in Petri dishes. Those which did not do so well at 25° were tried at 35° C. Each species was tested for 100 per cent germination. In the preliminary tests, 10 seeds of each species were used. In some instances the lemma and the palea were not removed in the first tests, hence it was not certain that there were seeds present. Afterward it was made a point that all protective bracts be removed from the seeds. Exceptions were made in the case of the *Bouteloua* grasses, in which the writer could find no seeds; hence the entire florets were used in hopes that some might contain seeds.

The *Aristida* seeds were tested for germination in the light, as they were supposed to show better germination results there than in the dark. The *Sporobolus* seeds were treated in several ways. The seed coats of nos. 9, 12, 14 were pricked, and the seeds shaken for 4, 6, and 9 hours in bottles containing coarse white sand, in order to injure the seed coats, thus hastening germination through the more easy entrance of water into the seed. Some of the seeds of nos. 9, 12, 14, which had been shaken for various lengths of time, were planted in sandy loam and kept at room temperature to see whether they would germinate more readily after having their seed coats bruised. Nos. 9, 12, 14, 22, 23 were soaked in distilled water for a period of 4 days and then for one of 9 days. These seeds were then placed in the 5° C. oven for 7 days and 21 days, and then in the 25° C. germinating oven. Nos. 4 and 20 were treated with varying solutions of CaCO<sub>3</sub>. Ten seeds of all the grasses, excepting the *Bouteloua* species, were planted in sandy loam.

TABLE I A  
TABULAR DESCRIPTION OF SEEDS COLLECTED BY R. S. CAMPBELL

SEED NO.	SCIENTIFIC NAME	COMMON NAME	GEOGRAPHIC RANGE	PLACE OF COLLECTION	DATE OF COLLECTION (1936)
1.	<i>Hilaria mutica</i>	Tabosa grass	Western Texas to southern Arizona and adjacent Mexico	1½ m. southwest of Red Lake Well	Sept. 19
2.	<i>Hilaria mutica</i>	Tabosa grass	Western Texas to southern Arizona and adjacent Mexico	1 m. south of Middle Well	Sept. 1
3.	<i>Muhlenbergia porteri</i>	Porter's bush grama	Colorado and western Texas to California and Mexico	2 m. south of Taylor Well	.....
4.	<i>Sporobolus airoides</i>	Alkali sacaton	Washington and Nebraska to California and New Mexico	1½ m. northeast of Hdqrs.	Aug. 31
5.	<i>Aristida purpurea</i>	Purple three-awn	Southwestern U.S. to southern Mexico	1½ m. northeast of Hdqrs.	Aug. 31
6.	<i>Scleropogon brevifolius</i>	Burro grass	Arizona and western Texas to Mexico and South America	¼ m. northeast of Hdqrs.	Aug. 31
7.	<i>Bouteloua eriopoda</i>	Black grama grass	Arizona and western Texas to Mexico	1½ m. northeast of Hdqrs.	Aug. 31
8.	<i>Muhlenbergia arenicola</i>	Ring muhlenbergia	Colorado and Kansas to Texas and New Mexico	1½ m. northeast of Hdqrs.	Aug. 31
9.	<i>Sporobolus cryptandrus</i>	Sand grass	Washington and Maine to Arizona and Texas	1½ m. northeast of Hdqrs.	Aug. 31
10.	<i>Bouteloua gracilis</i>	Blue grama grass	Manitoba to Mexico, and even to South America	St. Nicholas Canyon enclosure	Sept. 1
11.	<i>Bouteloua eriopoda</i>	Black grama grass	Arizona and western Texas to Mexico	Enclosure no. 10	Nov. 9
12.	<i>Sporobolus giganteus</i>	Gigantic sand grass	Southern New Mexico	1½ m. northeast of Hdqrs.	Sept. 19
13.	<i>Aristida longiseta</i>	Red three-awn grass	Southwestern U.S. and New Mexico	Aristida enclosure	Sept. 10
14.	<i>Sporobolus flexuosus</i>	Wide-panicked grass	Nevada to Texas and Mexico	1½ m. northeast of Hdqrs.	Aug. 31
15.	<i>Aristida longiseta</i>	Red three-awn grass	Southwestern U.S. and northern Mexico	1½ m. northeast of Hdqrs.	Aug. 31
16.	<i>Bouteloua curtipendula</i>	Side oats grama grass	Canada to New Jersey, California, and Mexico	Lion Den Canyon	Sept. 1
17.	<i>Sporobolus auriculatus</i>	Dwarf dropseed	Western Texas to southern New Mexico	¼ m. north of Hdqrs.	Aug. 31

TABLE IB  
TABULAR DESCRIPTION OF SEEDS

SEED NO.	SCIENTIFIC NAME	ALTITUDE OF PLACE OF COLLECTION (FEET)	TYPE OF SOIL	RAINFALL				PERCENT-AGE DISSEMINATION	PERCENT-AGE GERMINATION
				SEASONAL		ANNUAL			
				Inches	Zone	Inches	Zone		
1.....	<i>Hilaria mutica</i>	4200	Heavy clay	4.82	1	14.67	1	.....	34
2.....	<i>Hilaria mutica</i>	4200	Low swag heavy clay	7.51	4	18.29	5	.....	91
3.....	<i>Muhlenbergia porteri</i>	4050	Gravelly clay-sand	10.24	6	19.48	5	.....	75
4.....	<i>Sporobolus airoides</i>	4200	Clay loam	9.64	6	17.42	4	.....	92
5.....	<i>Aristida purpurea</i>	4200	Sandy loam	9.64	6	17.42	4	.....	60
6.....	<i>Scleropogon brevifolius</i>	4200	Clay loam	8.53	5	17.42	4	5	46
7.....	<i>Bouteloua eriopoda</i>	4200	Sandy loam	9.64	6	17.42	4	.....	.....
8.....	<i>Muhlenbergia arenicola</i>	4200	Clay loam	9.64	6	17.42	4	.....	60
9.....	<i>Sporobolus cryptandrus</i>	4200	Sandy loam	9.64	6	17.42	4	.....	75
10.....	<i>Bouteloua gracilis</i>	5600	Gravelly loam	11.23	8	21.17	7	5	.....
11.....	<i>Bouteloua eriopoda</i>	4050	Gravelly loam	7.65	4	17.42	4	.....	.....
12.....	<i>Sporobolus giganteus</i>	4200	Sandy loam	9.64	6	17.42	4	.....	97
13.....	<i>Aristida longiseta</i>	4300	Very sandy loam	5.73	2	18.53	2	25	17
14.....	<i>Sporobolus flexuosus</i>	4200	Sandy loam	9.64	6	17.42	4	30	42
15.....	<i>Aristida longiseta</i>	4200	Sandy loam	9.64	6	17.42	4	.....	53
16.....	<i>Bouteloua curtipendula</i>	4800	Gravelly loam	11.23	7	21.17	6	10	98.6
17.....	<i>Sporobolus auriculatus</i>	4100	Clay loam	8.53	5	17.42	4	.....	100

TABLE II

PRELIMINARY TEST ON BLOTING PAPER, 25° C.; JANUARY 19-FEBRUARY 18, 1927  
(31 DAYS)

No.	NAME	NO. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
1.....	<i>Hilaria mutica</i>	10	1/27	10
2.....	<i>Hilaria mutica</i>	10	1/27	60
3.....	<i>Muhlenbergia porteri</i>	10	1/27	10
4.....	<i>Sporobolus airoides</i>	10	1/24	40
5.....	<i>Aristida purpurea</i>	9	1/24	33.3
6.....	<i>Scleropogon brevifolius</i>	10	2/3	20
7.....	<i>Bouteloua eriopoda</i>	10*	.....	.....
8.....	<i>Muhlenbergia arenicola</i>	10	2/3	30
9.....	<i>Sporobolus cryptandrus</i>	10	2/10	30
10.....	<i>Bouteloua gracilis</i>	10*	.....	.....
11.....	<i>Bouteloua eriopoda</i>	10*	.....	.....
12.....	<i>Sporobolus giganteus</i>	10	2/10	50
13.....	<i>Aristida longiseta</i>	10	.....	Moldy
14.....	<i>Sporobolus flexuosus</i>	10	2/15	80
15.....	<i>Aristida longiseta</i>	10	1/24	50
16.....	<i>Bouteloua curtipendula</i>	10	.....	Moldy
17.....	<i>Sporobolus auriculatus</i>	10	1/27	20

\* Empty lemmas.

TABLE III

PRELIMINARY TESTS ON BLOTING PAPER, 25° C.; FEBRUARY 4-25, 1927 (22 DAYS)

No.	NAME	NO. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
1.....	<i>Hilaria mutica</i>	10	2/7	20
2.....	<i>Hilaria mutica</i>	10	2/7	100
3.....	<i>Muhlenbergia porteri</i>	10	2/7	80
4.....	<i>Sporobolus airoides</i>	10	2/15	20
5.....	<i>Aristida purpurea</i>	10	2/10	70
6.....	<i>Scleropogon brevifolius</i>	10	2/25	10 (9 moldy)
7.....	<i>Bouteloua eriopoda</i>	10*	.....	.....
8.....	<i>Muhlenbergia arenicola</i>	10	2/10	30
9.....	<i>Sporobolus cryptandrus</i>	10	2/15	60
10.....	<i>Bouteloua gracilis</i>	10*	.....	.....
11.....	<i>Bouteloua eriopoda</i>	10*	.....	.....
12.....	<i>Sporobolus giganteus</i>	10	2/25	40
13.....	<i>Aristida longiseta</i>	10	2/7	30
14.....	<i>Sporobolus flexuosus</i>	10	2/25	30
15.....	<i>Aristida longiseta</i>	10	2/7	40
16.....	<i>Bouteloua curtipendula</i>	10	2/7	100
17.....	<i>Sporobolus auriculatus</i>	10	2/7	20

\* Empty lemmas.

TABLE IV

PRELIMINARY TEST IN PETRI DISH, 25° C.; JANUARY 27—FEBRUARY 25 (30 DAYS)

No.	NAME	No. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
1.....	<i>Hilaria mutica</i>	10	2/1	40
2.....	<i>Hilaria mutica</i>	10	1/29	100
3.....	<i>Muhlenbergia porteri</i>	10	2/1	60
4.....	<i>Sporobolus airoides</i>	10	1/29	70
5.....	<i>Aristida purpurea</i>	10	1/29	80
6.....	<i>Scleropogon brevifolius</i>	10	.....	Moldy
7.....	<i>Bouteloua eriopoda</i>	10*	.....	.....
8.....	<i>Muhlenbergia arenicola</i>	10	2/1	40
9.....	<i>Sporobolus cryptandrus</i>	10	1/22	40
10.....	<i>Bouteloua gracilis</i>	10*	.....	.....
11.....	<i>Bouteloua eriopoda</i>	10*	.....	.....
12.....	<i>Sporobolus giganteus</i>	10	1/22	40
13.....	<i>Aristida longiseta</i>	10	2/1	10
14.....	<i>Sporobolus flexuosus</i>	10	2/22	70
15.....	<i>Aristida longiseta</i>	10	2/1	80
16.....	<i>Bouteloua curtipendula</i>	10	2/3	100
17.....	<i>Sporobolus auriculatus</i>	9	2/1	100

\* Empty lemmas.

TABLE V

PRELIMINARY TEST IN PETRI DISH, 35° C.; FEBRUARY 2-16 (14 DAYS)

No.	NAME	No. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
1.....	<i>Hilaria mutica</i>	10	2/7	40
2.....	<i>Hilaria mutica</i>	Not tried	.....	.....
3.....	<i>Muhlenbergia porteri</i>	Not tried	.....	.....
4.....	<i>Sporobolus airoides</i>	Not tried	.....	.....
5.....	<i>Aristida purpurea</i>	Not tried	.....	.....
6.....	<i>Scleropogon brevifolius</i>	10	.....	0
7.....	<i>Bouteloua eriopoda</i>	10*	.....	0
8.....	<i>Muhlenbergia arenicola</i>	5	.....	0
9.....	<i>Sporobolus cryptandrus</i>	10	.....	0
10.....	<i>Bouteloua gracilis</i>	10*	.....	0
11.....	<i>Bouteloua eriopoda</i>	10*	.....	0
12.....	<i>Sporobolus giganteus</i>	10	.....	0
13.....	<i>Aristida longiseta</i>	10	2/7	10
14.....	<i>Sporobolus flexuosus</i>	10	.....	0
15.....	<i>Aristida longiseta</i>	Not tried	.....	.....
16.....	<i>Bouteloua curtipendula</i>	10	2/7	100
17.....	<i>Sporobolus auriculatus</i>	Not tried	.....	.....

\* Empty lemmas.

TABLE VI

PRELIMINARY TEST IN PETRI DISH, 25° C.; FEBRUARY 12-MARCH 15 (32 DAYS)

No.	NAME	No. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
1. ....	<i>Hilaria mutica</i>	10	2/15	20
2. ....	<i>Hilaria mutica</i>	10	2/15	80
3. ....	<i>Muhlenbergia porteri</i>	10	2/15	10
4. ....	<i>Sporobolus airoides</i>	10	2/17	90
5. ....	<i>Aristida purpurea</i>	10	2/15	40
6. ....	<i>Scleropogon brevifolius</i>	10	2/15	40
7. ....	<i>Bouteloua eriopoda</i>	10*	.....	.....
8. ....	<i>Muhlenbergia arenicola</i>	4	2/15	50
9. ....	<i>Sporobolus cryptandrus</i>	10	2/15	100
10. ....	<i>Bouteloua gracilis</i>	10*	.....	.....
11. ....	<i>Bouteloua eriopoda</i>	10*	.....	.....
12. ....	<i>Sporobolus giganteus</i>	10	2/15	100
13. ....	<i>Aristida longiseta</i>	10	2/15	10
14. ....	<i>Sporobolus flexuosus</i>	10	2/15	60
15. ....	<i>Aristida longiseta</i>	10	2/15	60
16. ....	<i>Bouteloua curtipendula</i>	10	2/15	100
17. ....	<i>Sporobolus auriculatus</i>	9	2/15	33.3

\* Empty lemmas.

TABLE VII

100 PER CENT GERMINATION TEST IN PETRI DISH, 25° C.; FEBRUARY 21-MARCH 16  
(24 DAYS)

No.	NAME	No. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
1. ....	<i>Hilaria mutica</i>	50	2/28	34
2. ....	<i>Hilaria mutica</i>	100	2/25	91
3. ....	<i>Muhlenbergia porteri</i>	20	2/28	75
4. ....	<i>Sporobolus airoides</i>	100	2/25	92
5. ....	<i>Aristida purpurea</i>	100	2/25	60
6. ....	<i>Scleropogon brevifolius</i>	50	2/28	48
7. ....	<i>Bouteloua eriopoda</i>	100*	.....	.....
8. ....	<i>Muhlenbergia arenicola</i>	20	2/25	60
9. ....	<i>Sporobolus cryptandrus</i>	100	2/25	75
10. ....	<i>Bouteloua gracilis</i>	100*	.....	.....
11. ....	<i>Bouteloua eriopoda</i>	100*	.....	.....
12. ....	<i>Sporobolus giganteus</i>	100	2/25	97
13. ....	<i>Aristida longiseta</i>	100	2/25	17
14. ....	<i>Sporobolus flexuosus</i>	100	2/25	42
15. ....	<i>Aristida longiseta</i>	100	2/25	53
16. ....	<i>Bouteloua curtipendula</i>	75	2/28	98.6
17. ....	<i>Sporobolus auriculatus</i>	25	2/28	100

\* Empty lemmas.

TABLE VIII

PRELIMINARY TEST ON BLOTTING PAPER, 25° C.; JANUARY 19—FEBRUARY 18 (31 DAYS)

No.	NAME	No. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
20.....	<i>Sporobolus airoides</i>	10	1/29	100
21.....	<i>Sporobolus auriculatus</i>	10*	.....	.....
22.....	<i>Sporobolus cryptandrus</i>	10	2/10	80
23.....	<i>Sporobolus flexuosus</i>	10	2/10	60
24.....	<i>Epicampes emersleyi</i>	10*	.....	.....
25.....	<i>Muhlenbergia arenicola</i>	10	2/1	30

\* Empty lemmas.

TABLE IX

PRELIMINARY TEST IN PETRI DISH, 25° C.; JANUARY 27—FEBRUARY 25 (30 DAYS)

No.	NAME	No. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
20.....	<i>Sporobolus airoides</i>	10	2/1	100
21.....	<i>Sporobolus auriculatus</i>	Empty lemmas	.....	.....
22.....	<i>Sporobolus cryptandrus</i>	10	2/22	70
23.....	<i>Sporobolus flexuosus</i>	10	2/22	80
24.....	<i>Epicampes emersleyi</i>	Empty lemmas	.....	.....
25.....	<i>Muhlenbergia arenicola</i>	10	2/1	40
26.....	<i>Panicum obtusum</i>	10	2/7	30

TABLE X

PRELIMINARY TEST IN PETRI DISH, 35° C.; FEBRUARY 2—16 (14 DAYS)

No.	NAME	No. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
20.....	<i>Sporobolus airoides</i>	Not tried	.....	.....
21.....	<i>Sporobolus auriculatus</i>	5	2/10	20
22.....	<i>Sporobolus cryptandrus</i>	10	.....	.....
23.....	<i>Sporobolus flexuosus</i>	10	.....	.....
24.....	<i>Epicampes emersleyi</i>	Empty lemmas	.....	.....
25.....	<i>Muhlenbergia arenicola</i>	9	2/7	77.8
26.....	<i>Panicum obtusum</i>	10	.....	.....

TABLE XI

100 PER CENT GERMINATION TEST IN PETRI DISH, 25° C.; FEBRUARY 21-MARCH 16  
(24 DAYS)

No.	NAME	NO. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
20.....	<i>Sporobolus airoides</i>	100	2/25	92
21.....	<i>Sporobolus auriculatus</i>	6	2/25	100
22.....	<i>Sporobolus cryptandrus</i>	20	2/25	95
23.....	<i>Sporobolus flexuosus</i>	40	2/25	92.5
24.....	<i>Epicampes emersleyi</i>	Not tried	.....	.....
25.....	<i>Muhlenbergia arenicola</i>	40	2/25	90
26.....	<i>Panicum obtusum</i>	100	2/28	21

TABLE XII

GERMINATION IN LIGHT IN PETRI DISH, ROOM TEMPERATURE, ROOM LIGHT;  
MARCH 1-16 (16 DAYS)

No.	NAME	NO. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
5.....	<i>Aristida purpurea</i>	10	3/5	40
13.....	<i>Aristida longiseta</i>	10	3/5	20
15.....	<i>Aristida longiseta</i>	10	3/5	80

TABLE XIII

SEEDS SOAKED IN DISTILLED H<sub>2</sub>O 4 DAYS, THEN IN GERMINATING OVEN AT 25° C.  
FOR 2 WEEKS

No.	NAME	NO. USED	GERMINATION DURING SOAKING	GERMINATION AFTER SOAKING	PERCENTAGE GERMINATION
9.....	<i>Sporobolus cryptandrus</i>	15	0	0	0
12.....	<i>Sporobolus giganteus</i>	15	0	0	6.6
14.....	<i>Sporobolus flexuosus</i>	15	0	0	0
20.....	<i>Sporobolus airoides</i>	40	34	0	85
22.....	<i>Sporobolus giganteus</i>	15	0	0	0
23.....	<i>Sporobolus flexuosus</i>	15	0	0	0



TABLE XIV

SEEDS SOAKED IN DISTILLED H<sub>2</sub>O 10 DAYS, THEN IN GERMINATING OVEN AT 25° C.  
FOR 2 WEEKS

No.	NAME	NO. USED	GERMINATION DURING SOAKING	GERMINATION AFTER SOAKING	PERCENTAGE GERMINATION
9.....	<i>Sporobolus cryptandrus</i>	15	0	2	13.3
12.....	<i>Sporobolus giganteus</i>	15	2	0	13.3
14.....	<i>Sporobolus flexuosus</i>	15	1	0	6.6
20.....	<i>Sporobolus airoides</i>	15	12	0	80
22.....	<i>Sporobolus giganteus</i>	15	0	1	6.6
23.....	<i>Sporobolus flexuosus</i>	15	1	0	6.6

TABLE XV

SEEDS SHAKEN IN COARSE WHITE SAND, THEN IN 25° C. GERMINATING  
OVEN FOR 2 WEEKS

No.	NAME	NO. USED	NO. OF HOURS SHAKEN	NO. OF DAYS SOAKED	PERCENTAGE GERMINATION
9.....	<i>Sporobolus cryptandrus</i>	10	6	5	10
12.....	<i>Sporobolus giganteus</i>	10	6	5	10
14.....	<i>Sporobolus flexuosus</i>	10	6	5	10

TABLE XVI

SEEDS SHAKEN IN COARSE WHITE SAND, THEN PLACED IN SANDY LOAM

No.	NAME	NO. USED	NO. OF HOURS SHAKEN	PERCENT- AGE GERMINA- TION	NO. OF HOURS SHAKEN	PERCENT- AGE GERMINA- TION
9.....	<i>Sporobolus cryptandrus</i>	10	6	0	9	0
12.....	<i>Sporobolus giganteus</i>	10	6	0	9	0
14.....	<i>Sporobolus flexuosus</i>	10	6	0	9	0

TABLE XVII

SEEDS IN GERMINATING OVEN AT 25° C. FOR 21 DAYS

No.	NAME (10 SEEDS OF EACH SPECIES USED)	PERCENTAGE GERMINATION
Without shaking or soaking (control)		
9.....	<i>Sporobolus cryptandrus</i>	10
12.....	<i>Sporobolus giganteus</i>	20
14.....	<i>Sporobolus flexuosus</i>	10
Shaken four hours		
9.....	<i>Sporobolus cryptandrus</i>	0
12.....	<i>Sporobolus giganteus</i>	0
14.....	<i>Sporobolus flexuosus</i>	0
Shaken six hours		
9.....	<i>Sporobolus cryptandrus</i>	10
12.....	<i>Sporobolus giganteus</i>	10
14.....	<i>Sporobolus flexuosus</i>	10
Shaken nine hours		
9.....	<i>Sporobolus cryptandrus</i>	10
12.....	<i>Sporobolus giganteus</i>	20
14.....	<i>Sporobolus flexuosus</i>	10

TABLE XVIII

No.	NAME	No. USED	PERCENTAGE GERMINATION	
			5° C. oven 7 days	25° C. oven 21 days
9.....	<i>Sporobolus cryptandrus</i>	15	0	0
12.....	<i>Sporobolus giganteus</i>	15	13.3	20
14.....	<i>Sporobolus flexuosus</i>	15	0	0
22.....	<i>Sporobolus cryptandrus</i>	15	0	0
23.....	<i>Sporobolus flexuosus</i>	15	0	0

TABLE XIX

SEEDS TREATED WITH  $\text{CaCO}_3$  SOLUTIONS INSTEAD OF DISTILLED  $\text{H}_2\text{O}$ 

No.	NAME	No. used	PERCENTAGE GERMINATION			
			0.5 per cent $\text{CaCO}_3$	1 per cent $\text{CaCO}_3$	5 per cent $\text{CaCO}_3$	10 per cent $\text{CaCO}_3$
4....	<i>Sporobolus airoides</i> (1926)	10	30	70	0	0
20....	<i>Sporobolus airoides</i> (1925)	15	87	80	0	0

TABLE XX

NO SEED SELECTION MADE; GLUMES PLACED IN PETRI DISHES  
AND THEN IN 25° C. OVEN

No.	NAME	RESULTS
7.....	<i>Bouteloua eriopoda</i>	No signs of germination
10.....	<i>Bouteloua gracilis</i>	No signs of germination
11.....	<i>Bouteloua eriopoda</i>	No signs of germination

TABLE XXI

SEEDS PLANTED IN SANDY LOAM AND KEPT AT ROOM TEMPERATURE (70° F.)  
FEBRUARY 28, 1927

No.	NAME	No. USED	DATE OF FIRST PLANT
1.....	<i>Hilaria mutica</i>	10	.....
2.....	<i>Hilaria mutica</i>	10	3/16
3.....	<i>Muhlenbergia porteri</i>	10	.....
4.....	<i>Sporobolus airoides</i>	10	.....
5.....	<i>Aristida purpurea</i>	10	.....
6.....	<i>Scleropogon brevifolius</i>	6	5/4
8.....	<i>Muhlenbergia arenicola</i>	10	.....
9.....	<i>Sporobolus cryptandrus</i>	10	5/5
12.....	<i>Sporobolus giganteus</i>	10	4/13
13.....	<i>Aristida longiseta</i>	10	.....
14.....	<i>Sporobolus flexuosus</i>	10	4/14
15.....	<i>Aristida longiseta</i>	10	.....
16.....	<i>Bouteloua curtipendula</i>	10	.....
17.....	<i>Sporobolus auriculatus</i>	10	.....

TABLE XXII  
PRECIPITATION JORNADA RANGE RESERVE, 1926

STATION	JANU- ARY	FEBRU- ARY	MARCH	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEM- BER	OCTO- BER	NOVEM- BER	DECEM- BER	SEASON- AL	ANNUAL
Headquarters . . . . .	0.49	0.05	1.40	0.48	2.43	0.06	4.95	0.38	3.20	2.32	0.04	1.62	8.53	17.42
Midwell . . . . .	0.56	0.01	2.28	0.59	3.09	0.01	3.21	1.06	3.24	2.23	0.04	1.97	7.51	18.29
Red Lake . . . . .	0.49	0.01	1.01	0.74	2.97	0.02	1.85	0.42	2.55	1.75	0.02	1.94	4.82	14.67
Road Tank . . . . .	0.01	0.01	1.82	0.67	1.71	0.00	5.10	0.22	3.91	2.37	0.06	1.78	0.32	18.35
Ropes . . . . .	0.84	0.12	2.65	0.88	2.71	0.40	2.52	1.09	4.11	2.36	0.00	2.00	7.72	19.68
St. Nicholas . . . . .	0.90	0.10	2.02	0.53	1.04	0.03	6.07	1.20	3.95	2.07	0.02	2.03	11.23	21.17
South Well . . . . .	0.53	0.00	1.57	0.91	2.09	T	4.01	0.25	3.48	2.61	0.01	1.84	7.74	17.30
Stuart Well . . . . .	0.55	0.00	1.97	0.71	1.93	T	4.75	0.25	2.78	2.66	0.04	1.73	7.78	17.37
Ragged Well . . . . .	0.43	0.00	1.06	1.00	2.21	0.09	6.30	1.16	2.78	2.24	0.00	1.31	10.24	19.48
West Well . . . . .	0.55	0.00	1.66	0.71	1.98	T	2.48	1.66	4.81	2.53	0.12	2.03	8.95	19.53
Period Study . . . . .	.....	.....	1.62	0.51	2.00	0.01	5.42	0.52	3.70	2.52	0.00	1.89	9.64	.....
Enclosure no. 1 . . . . .	.....	.....	1.51	0.68	2.45	0.00	4.00	0.25	3.04	2.35	0.00	2.05	7.89	.....
Enclosure no. 10 . . . . .	.....	.....	1.37	0.88	1.94	0.02	3.81	0.31	3.53	2.34	0.00	2.00	7.65	.....
Enclosure no. 2 . . . . .	.....	.....	.....	.....	.....	.....	2.11	0.47	4.16	2.43	0.01	1.78	6.74	.....
Aristida . . . . .	.....	.....	.....	.....	.....	.....	2.77	0.42	2.54	0.89	0.79	2.01	5.73	.....
Brown Tank . . . . .	.....	.....	.....	.....	.....	.....	5.76	0.04	3.21	2.30	0.03	1.36	0.01	.....
Sand Hills . . . . .	.....	.....	.....	.....	.....	.....	5.24	0.39	3.15	2.09	0.00	1.98	8.68	.....
New Well . . . . .	.....	.....	.....	.....	.....	.....	5.76	0.81	3.58	1.84	0.40	1.95	10.15	.....
Average . . . . .	0.59	0.03	1.92	0.72	2.28	0.06	4.23	0.60	3.46	2.25	0.13	1.85	8.29	18.23

TABLE XXIII

COMPARISON OF SEASONAL RAINFALL FOR (A) *HILARIA MUTICA* NO. 1 AND NO. 2;  
(B) *ARISTIDA LONGISETA* NO. 13 AND NO. 15\*

PRECIPITATION (INCHES)			
(a) <i>Hilaria mutica</i> , no. 1		<i>Hilaria mutica</i> , no. 2	
July.....	1.85	July.....	3.21
August.....	0.42	August.....	1.06
September (19 days).....	1.61		
Total.....	3.88	Total.....	4.27
(b) <i>Aristida longiseta</i> , no. 13		<i>Aristida longiseta</i> , no. 15	
July.....	2.77	July.....	5.42
August.....	0.42	August.....	0.52
September (16 days).....	1.52		
Total.....	4.71	Total.....	5.94

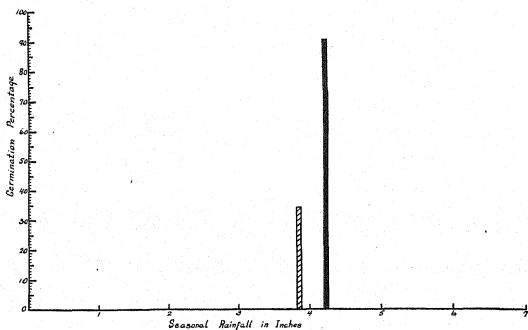
\* See figs. 1, 2, 3.

TABLE XXIV

SEASONAL PRECIPITATION ON JORNADA RANGE RESERVE JULY, AUGUST,  
AND SEPTEMBER, 1926

No.	STATION	PRECIPITATION (INCHES)	RANGE (INCHES)
1.....	Red Lake	4.82	Under 5
2.....	Aristida Enclosure	5.73	5-6
3.....	Enclosure no. 2	6.74	6-7
4.....	Middle Well	7.51	7-8
5.....	Enclosure no. 10	7.65	7-8
6.....	Ropes	7.72	7-8
7.....	South Well	7.74	7-8
8.....	Stuart Well	7.78	7-8
9.....	Enclosure no. 1	7.89	7-8
10.....	Headquarters	8.53	8-9
11.....	Sand Hills	8.68	8-9
12.....	West Well	8.95	8-9
13.....	Brown Tank	9.01	9-10
14.....	Road Tank	9.32	9-10
15.....	Period Study	9.64	9-10
16.....	New Well	10.15	10-11
17.....	Ragged Tank	10.24	10-11
18.....	St. Nicholas	11.23	Over 11

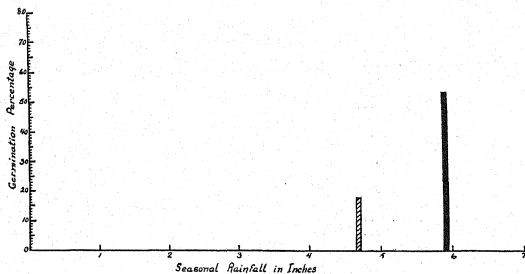
The areas allotted to the various stations are based on the records of the individual stations, supplemented by general observation throughout the year. Fig. 4 shows the approximate area covered by the different amounts of rainfall.



Comparison of Germination Percentages of  
*Hilaria mutica* No. 1 and No. 2

Legend	<i>Hilaria mutica</i> No. 1	<i>Hilaria mutica</i> No. 2
	Seasonal rainfall 3.88 inches	Seasonal rainfall 4.27 inches
	Germination 34%	Germination 91%

FIG. 1



Comparison of Germination Percentages of  
*Aristida longiseta* No. 13 and No. 15

Legend	<i>Aristida longiseta</i> No. 13	<i>Aristida longiseta</i> No. 15
	Seasonal rainfall 4.71 inches	Seasonal rainfall 5.94 inches
	Germination 17%	Germination 54%

FIG. 2

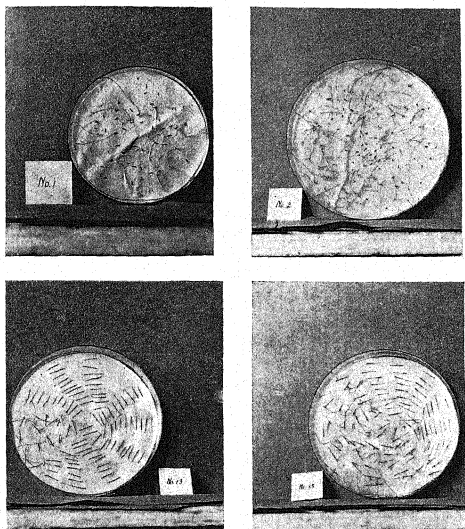


FIG. 3.—Upper row: comparison of germination of *Hilaria mutica* no. 1 (50 seeds, 34 per cent germination) and no. 2 (100 seeds, 91 per cent germination); lower row: comparison of germination of *Aristida longiseta* no. 13 (100 seeds, 17 per cent germination) and no. 15 (100 seeds, 54 per cent germination).

# Seasonal Precipitation Map for 1926

The Jornada Range Reserve  
Doña Ana County  
New Mexico

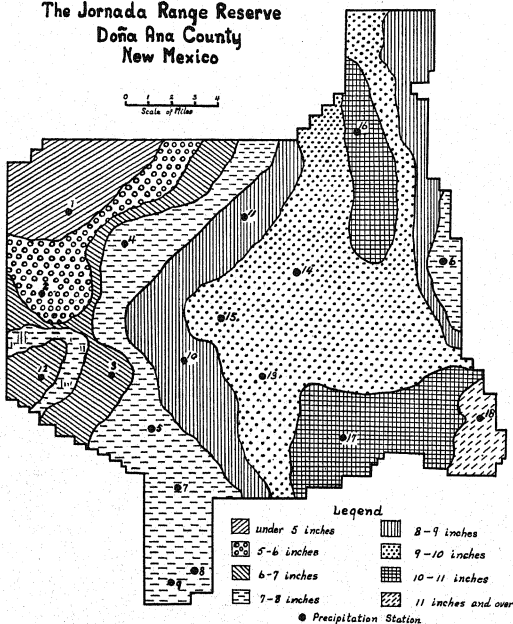


FIG. 4



# Annual Precipitation Map for 1926

The Jornada Range Reserve  
Doña Ana County  
New Mexico

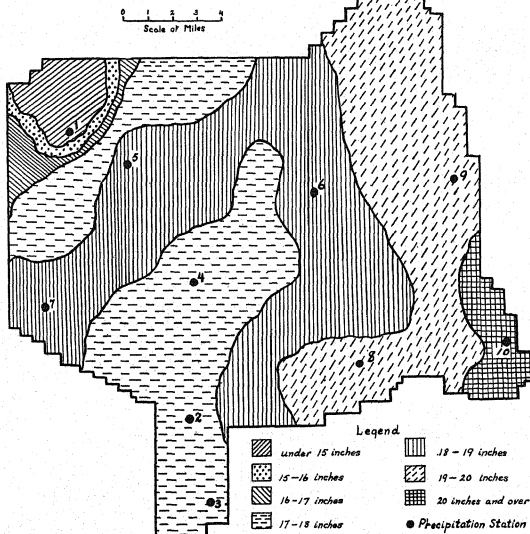


FIG. 5

The areas allotted to the various stations are based on the records of the individual stations, supplemented by general observation throughout the year. Fig. 5 shows the approximate areas covered by the different amounts of rainfall.

TABLE XXV  
ANNUAL PRECIPITATION ON JORNADA RANGE RESERVE, 1926

No.	STATION	PRECIPITATION (INCHES)	RANGE (INCHES)
1.....	Red Lake	14.67	Under 15 15-16 16-17
2.....	South Well	17.30	17-18
3.....	Stuart Well	17.39	17-18
4.....	Headquarters	17.42	17-18
5.....	Middle Well	18.29	18-19
6.....	Road Tank	18.33	18-19
7.....	West Well	18.83	18-19
8.....	Ragged Tank	19.48	19-20
9.....	Ropes	19.68	19-20
10.....	St. Nicholas	21.17	Over 20

### Discussion

From the tables of the various tests can be ascertained the germination percentages under different conditions. Table VII gives the final germination percentage for the seeds collected in 1926 and table IX for those gathered in 1925. From all the results it may be seen that the two lots of *Hilaria mutica* (nos. 1, 2) show a decided difference in their germination percentages. In no. 1 the germination percentage is always lower than in no. 2. By referring to the annual and seasonal rainfall figures, especially to the seasonal which includes just the rainfall during July, August, and September, when growth is taking place, one finds that no. 2 was collected in an area which received 2.69 inches more for the whole year than did the area in which no. 1 was growing. This probably accounts for the difference in the germination percentage, even though no. 2 was collected 19 days sooner than no. 1. The same is true of *Aristida longiseta* nos. 13 and 15. No. 15 was collected 16 days sooner than no. 13, but it gives much better germination results. The seeds of no. 15 received 1.23 inches more rainfall during the growing season than did the no. 13 seeds.

The *Bouteloua* (*B. eriopoda* and *B. gracilis*) had no seeds, so there was no germination percentage for either of them. Although tests were made in which the entire spikes were placed in sterilized Petri dishes in the 25° C. oven, no germination results were obtained. It is known that the *Bouteloua* grasses seed rarely, their means of propagation being vegetative rather than sexual.

Tables V and X show that germination is not increased by higher temperatures, as 35° C., nor does the alternation of low and high temperatures, that is from 5 to 25° C., increase germination (table XVIII). The best results are at 25° C.

In regard to the *Sporobolus* seeds nos. 9, 12, 14, 22, and 23, which have an unusually hard seed coat, it was found necessary to prick them in order to obtain germination results. Shaking the seeds in bottles containing coarse sand helped germination somewhat, but the writer believes that much better results could be obtained if the period of shaking were considerably extended. This method would scratch the coats of the seeds in a way similar to that in nature.

### Summary

1. The amount of rainfall during the year, especially during the growing season and at the time of harvesting, affects the vitality of the seed.

2. *Bouteloua eriopoda* and *B. gracilis* do not seed very often. Most of the florets are sterile, and because of their similarity to the fertile florets, are hard to distinguish from them.

3. *Aristida* seeds germinate just as well in the light as they do in the dark.

4. The seed coat is important in *Sporobolus* seeds, as it keeps out water and prevents germination. The seed coat must be punctured by some means before good germination results. Soaking affects the seed coats but little; shaking even for 9 hours in sand has little effect; and scratching or pricking hastens germination greatly.

5. The seeds of *Sporobolus airoides* do not need pricking to produce good germination results. The seed coat is more permeable to water than are the seed coats of the other species.

6. The *Sporobolus* seeds from 1925 retained their vitality very well.

I wish to thank Mr. J. D. SCHOELLER, the Director of the Jornada Range Reserve Station, for supplying the seeds used in this investigation and for the climatic and geographic data of the region. I also wish to express my gratitude to Professor H. C. COWLES for his continued interest and suggestion throughout the work.

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CHROMOSOME STUDIES IN THE CYPERACEAE,  
WITH SPECIAL REFERENCE  
TO SCIRPUS<sup>1</sup>

G. CLAUDE HICKS

(WITH PLATES X, XI)

Introduction

The Cyperaceae are of interest because they present situations unique among the Angiosperms. In the development of their microspores, the pollen mother cells undergo the usual meiotic divisions, but of the four nuclei so produced only one develops a grain. Another striking feature, that parallels the unique mode of formation of microspores, lies in the chromosome numbers investigated to date. Within the last decade, HEILBORN has published a series of papers (18, 19, 20) on the chromosomes, chromosome numbers, chromosome dimensions, and species formation, in the very large genus *Carex*. In the 44 species investigated, he found some 22 different numbers, ranging from 9 to 56, and which did not come under the law of multiples, namely, 9, 15, 16, 19, 24, 25, 27, 28, 29, 31, 33, 34, 35, 36, 37, 38, 40, 41, 42, and 56.

While this condition, termed aneuploid (TÄCKHOLM 42, 43), also dysploid (JEFFREY 21), is prevalent in genera of both plants and animals, HEILBORN maintains that *Carex* occupies a singular position in this regard. He finds that the differences of size in the chromosomes are not proportional, in the forms that do manifest multiples. He thus raises the question of quality and not quantity, and because the size classes are represented in quite different proportions in the different species, he concludes that polyploidy does not exist in *Carex*.

In explanation of such a condition, he gives in substance a summary (19) of the influences which seem to him to have borne and bear on the evolution of the chromosome numbers. They are: (1) the probable transverse division of original large chromosomes; (2)

<sup>1</sup> Contributions from the Laboratories of Plant Morphology, Harvard University.

a slow gradual change from the lower to the higher numbers; (3) this gradual change brought about through the duplication of entire chromosomes; (4) the duplication which is most probably a result of failing conjugation between homologous pairs, the univalents thus produced arranging themselves in the equatorial plate in the meiotic divisions and then being divided; (5) the cause of the lacking conjugation which is unknown, but may have been the result of previous crosses (although it is unlikely that crossing has played any great rôle in the evolution of the chromosome groups in this genus, as *Carex* hybrids are largely sterile); (6) the evolution proceeding from lower numbers to higher ones.

As regards species formation, HEILBORN finds that mutations of *Oenothera lutea* type have been one of the most important processes, while hybridization can only be regarded as a factor rather inferior in importance.

In *Scirpus*, HAKANSSON (17) has reported the chromosome numbers 10, 13, 19, 21, 22, 23, 28, 29, 31, and 52. To explain this situation he suggests a transverse division of single chromosomes, and possibly a duplication of whole chromosomes, and is of the opinion that crossing may have been the cause of these numbers in some cases.

It accordingly seems profitable to study American material and other genera of the Cyperaceae for conditions, and thus to add to our cytological knowledge of chromosome number and behavior. The material which has been chosen would seem promising for this purpose, as it provides an excellent basis of comparison between stable and unstable species.

### Materials and methods

The material was collected as it grew, and the environs of metropolitan Boston provided unparalleled advantages for the study. The region has been thoroughly botanized by sedge lovers, the stations have been recorded in *Rhodora* and in publications dealing with the flora of the region, and they are usually easily reached. The Gray Herbarium, too, provides unequaled facilities for information as to stations and for checking plant determinations.

The gathering of material is not without difficulties, however

in spite of these advantages, as some of the species are very closely allied and are almost exclusively determined by the characters of the mature fruit; consequently the taxonomic phase is an unusual one. This is especially true where the species and varieties are most closely related. In some cases, however, the plants are fairly definite, and one is greatly helped by the recorded flowering season and regional distribution.

Since it is highly important to have mature material with which to determine that which was gathered in a green condition, two lines of approach are open: (1) the collection of mature plants in the previous season, marking the spot and returning to collect at the proper time; or (2) the collection of immature plants in a place that can be marked, and returning to gather the material when it is ripe. It is of course very necessary to know the plants before going into the field, in order that there may be proper discrimination.

The inflorescences were collected on warm days and put immediately into Carnoy's fluid, since comparative experiment justified the use of that liquid. The air was then drawn quickly from the tissues by means of an exhaust pump, to insure rapid fixation. Material gathered in the early afternoon gave more satisfactory results on the whole. After being left in the fixative for 24 hours, the plants were washed in from two to four changes of 95 per cent alcohol.

If the plants were imbedded immediately they would not cut satisfactorily, because of the mineralization of the tissues. Moreover, the cytoplasm of water plants often contains dark substances which interfere with proper staining, so that it is generally necessary to demineralize and bleach. A stock solution for such is made by adding crystals of sodium chlorate to strong hydrofluoric acid, until a saturated solution is obtained. The material is passed into water, and is then put into specially prepared waxed bottles containing a diluted solution of the fluid described, and left for only such time as is essential to the necessary degree of softening and bleaching.

Following this treatment, the material is dehydrated and imbedded in nitrocellulose, in which distortion is much less likely than in paraffin. This is the method described by JEFFREY (21). The writer has also used with advantage the mass method, devised in connection with work on *Drosophila melanogaster* (JEFFREY and

HICKS 23, 24) and later improved. With this method exceptionally quick results are obtained satisfactorily. A Jung-Thoma sliding microtome was used for cutting sections. Both longitudinal and transverse sections were cut 10 and 5  $\mu$  thick. It was demonstrated that transverse sections were best for details.

Haidenhain's iron haematoxylin was used for staining and gave satisfactory results. Slow staining and slow decolorizing over long periods imparted to the chromosomes an unusually dark aspect. Further, when the cytoplasm was stained over night in a very weak solution of eosin in 30 per cent alcohol, most excellent results in contrast were obtained.

The sections were then mounted in Canada balsam, and after the benzol had evaporated the cover slips were pressed down with small lead weights.

In the study and drawing of the material, a Bausch and Lomb microscope, equipped with a 140° Abbé condenser, was used. Since strong light and resolution were very important factors, a 3 mm. 140° Bausch and Lomb apochromatic lens, together with a no. 15 "periplan" compensating ocular, provided an excellent combination of equipment. The drawings were outlined from typical stages of the pollen mother cells by means of a camera lucida with the tube length of the microscope at 160. They were then enlarged three times, except where noted, with the aid of a no. 346 Starrat rule graduated to fifths of a millimeter, in order that the proportions be maintained.

Counts of the chromosomes were made mostly from the heterotypic metaphase plates, but, where possible, use was made of the diakinesis and homotypic stages.

### Observations

The chromosomes in some of the species are rather small, and it is difficult to know the exact conditions of affinity from a study of diakinesis. In many cases it seems best not to group the chromosomes into classes as no consistent results are obtained. Variations occurring in such cases may be due to the orientation of the chromosomes on the spindle. There is variation, however, among the chromosomes of each nucleus and between nuclei of the different species;



the *Scirpus lacustris* group has the largest chromosomes, while the *S. maritimus* group has the smallest. No successful attempts have been made to classify the chromosomes into different categories, as has been done in *Carex*. Haploid counts are recorded here.

*Scirpus heterochaetus* Chase.—Collections of this species were made along the Concord River at Bedford, and at Heard's Pond, Wayland, Massachusetts, and Cow Island Bay on the Charles River.

No early diakineses were seen. The size differences seen in the metaphase plates are not ordinarily evident. In fig. 1, 18 chromosomes are found as usual. These show size differences, one being conspicuously larger. In addition to the large chromosome, there are 6 of medium size and 11 of smaller size. The large chromosome may be quadrivalent, but it was not possible to demonstrate this condition. On one occasion there appeared to be 19 chromosomes.

The divisions are regular, excepting that the dark material (extranuclear chromatin) appears as it does in *S. validus*.

Mrs. CHASE (4), in defining the species, based its distinction on the three cleft styles, the triquetrous achene, fragile bristles, and glabrous scales. The solitary spikelets which she mentions are not always consistent features, however.

*Scirpus acutus* Muhl. f. *condensatus* (Farwell) Fern.—Mrs. CHASE comments that this species shows great variation regionally. A single collection proved to be this species, from an inspection of the material which was mature. This was gathered at the lower Mystic Lake. An unidentified collection from Cape Breton Island, Nova Scotia, showed the same results.

In the early stages of diakinesis the chromosomes are bivalent in character. In fig. 2 are 20 chromosomes lying in the metaphase plate. Of these one is conspicuously larger than the others. At times all the elements are round in form; at other times they show a constricted condition, and in this arrangement the large chromosome is quadripartite, a condition seen in late prophase also. It may be that this chromosome is quadrivalent.

The anaphase in the Mystic Lake material shows a lagging of the chromosomes, but no chromosomes are left in the cytoplasm. After this stage, the pollen mother cells are very much shrunken, and the condition persists through the homotypic division. Because of the

shrunken condition and the dark-staining capacity of the cells in this condition, the chromosomes cannot be followed any longer accurately.

The pollen is exceedingly bad, with disintegrating protoplasmic contents. The embryo sacs develop as usual.

*Scirpus validus* Vahl.—Individuals of this species were collected at Spot Pond, Nahant, Spy Pond, and on the Neponset River at Dedham Road Station, Massachusetts.

In diakinesis the nucleolus is rather large and is often constricted in outline rather than round. The chromosomes are to all appearances double, but at times perplexingly appear rounded off as single units.

The metaphase plates in all cases show 21 chromosomes (fig. 3); and although the chromosomes vary to some degree, there are no remarkable size differences, excepting that in Spot Pond material there is one large chromosome which is not always present in members of the same anther sac. They may be roughly classified as 7 of larger and 14 of smaller size.

The anaphases show themselves regular; in only one case was an irregularity seen. The chromosomes in going back to the poles do not manifest the paired condition. In the material from Spy Pond, some of the cells from the beginning of the telophases are very much collapsed.

In this species there is a vacuole in the apex of the pollen mother cell, and in this vacuole appears dark-staining material, such as is also found in the cytoplasm generally after the anaphase. KUWADA (28), describing such in *Zea mays*, writes of them as "extra-nuclear nucleoli." The same material appears in the cytoplasm before the anaphase, but at times it appears more pronounced after this stage.

The material persists throughout the homotypic divisions, which are quite regular. Often the perinuclear zones fuse and give an "8" effect. In interkinesis the chromosomes retain their form for some time. Cell plates sometimes appear as dark areas across the spindle fibers, and are not evident at times.

*Scirpus atrovirens* Muhl.—Only one collection proved to be of this species, and was made at Fresh Pond, Cambridge, Massachusetts, rather late in the season.

No suitable figures of diakinesis were found, and the metaphase plates were often not clear, due to clumping. In most cases a large double chromosome is present, with a second one at times. The number cannot be established definitely with the material at hand. Varying counts have been made, from 25 to 30 (fig. 4). This may be due to weakened affinity and may also be due to the fact that the chromosomes are hard to delimit.

Chromatin appears in the cytoplasm after the heterotypic, and for the most part the behavior of the chromosomes in the divisions is regular, with some very few cases of lagging. There are some cases, however, in which in the same anther sac are found heterotypic plates and telophases, interkinesis, and also homotypic metaphases. Cell plates are present in varying degrees.

One of the most prominent cases of cytomixis appears in this species (fig. 5). This phenomenon is seen in all stages, from synizesis to diakinesis, and renders accurate examination of the latter impossible.

The species has been found to develop green tufts of leaves from the axes of the inflorescence, after the achenes have begun to shed.

*Scirpus georgianus* Harper; *S. atrovirens* Muhl. var. *georgianus* (Harper) Fern.—Material of this variety was taken from two stations at Turtle Pond, Roxbury, one at Glacialis Pond, Cambridge, and one from Moose Hill, Sharon, Massachusetts.

All elements appear double in diakinesis, and never more than 28 were seen (fig. 6). The determination of the chromosome number is best accomplished in the metaphase plates, and this number has always been found to be the same. Cytomixis occurs up to the metaphase of the heterotypic divisions. The homotypic anaphases are perfectly regular. Faint and short-lived cell plates exist. In certain localities there are also found plants which have the tufts of leaves mentioned in connection with the preceding species.

*Scirpus rubrotinctus* Fern.—Material from Moncton, New Brunswick, merely provided examples of good pollen, while collections from Fresh Pond, Cambridge, and from Stoneham, Massachusetts, gave excellent cytological material for all stages desired.

Counts of diakinesis were not entirely satisfactory. There are al-

ways 33 chromosomes in the metaphase plates (fig. 7), 12 larger and 21 smaller, and the size differences can be recognized from a side view of the spindle.

*Scirpus longii* Fern.—After a great deal of searching for this species, a station was found in the Neponset River meadows at Dedham Road Station, Massachusetts.

This species does not appear in GRAY's *Manual*, as it was not described until 1911 by FERNALD (7). Its nearest relatives are the *S. atrocinctus* group, and it is distinguished by the early season in which it flowers, its reddish brown achenes, and its sticky bracts. A cross-section through the stem at the bracts shows a deposit of material on the stem. The plants are robust, and the heads are larger than those of *S. atrocinctus* and present a shaggy appearance. The anthers are very long for the group in which the species belongs.

The late prophases were of no use, and the divisions of the pollen mother cells provided only a basis for the gross descriptions (fig. 8). They were absolutely useless in the determination of the chromosome number, as the chromosomes had clumped. By good chance a clear plate of the heterotypic division of the megaspore mother cell was discovered, and there are 34 chromosomes.

In the anaphases the chromosomes usually go back to the poles quite regularly, but there have been a few cases where they come to the metaphase plate irregularly (fig. 9). Much dark-staining material is found in the cytoplasm.

*Scirpus atrocinctus* Fern.—Material gathered at North Branch Station and Lakeville, New Brunswick, at Manchester, New Hampshire, and at Wenham, Massachusetts, shows identical conditions. The collection made at Wenham displays a brownish cast to the inflorescence, and at first sight would be taken for *S. pedicellatus*, but from the darkish cast of the involucels was determined as belonging to the species under consideration.

In diakinesis the chromosomes all appear normal. There is at times an irregular arrangement of the chromosomes as they come to the metaphase plate (fig. 10). The 34 chromosomes are divided into sizes: 6 larger, 18 medium, and 10 smaller. In no cases have quadrivalent chromosomes been found. Dark-staining material appears in the cytoplasm, as noted for the other species. The homo-

typic division is very regular, and the abortion of the nuclei is as usual.

*Scirpus cyperinus* (L.) Kunth. var. *pelius* Fern.—Members of this variety were collected at Mystic Lake, at Fresh Pond, Cambridge, and at Lake Waban, Wellesley, Massachusetts. The results were the same.

The stages in diakinesis are unsatisfactory as there was a great deal of clumping. A study of the metaphase plates showed 33 chromosomes. Size differences were also noted. All stages of divisions are regular (figs. 11, 12). The forma *condensatus* (Fern.) Blake of this variety is the same as the preceding one. The true species was found at Massapoag Lake, Sharon, Massachusetts, but after much cutting no results were obtained.

*Scirpus americanus* Pers.—Material was gathered at Heard's Pond, Wayland, Massachusetts, and the Charles River marsh, and on the marshes at Hyannis.

In the late prophases the elements seem to be in a paired condition. The metaphase plates show (fig. 13) 38 chromosomes of different sizes. All the stages are fairly regular, although there is frequently lack of uniformity of the chromosomes in the anaphase. The material requires much care to differentiate it properly, and at times the cytoplasm possesses a muddy appearance. Further, after the heterotypic divisions the cells are very much shrunken.

*Scirpus olneyi* Gray.—One collection was made at Hyannis, Massachusetts.

The chromosomes are to all appearances paired in diakinesis, and in the metaphase plates 39 chromosomes (fig. 14) are found. In the anaphase of the heterotypic division (fig. 15) are cell plates of extraordinary size. These likewise appear in the homotypic division. No irregularities are found in the cytoplasm, except that there is the same shrunken condition as noted in the preceding species.

*Scirpus americanus* Pers. (irregular form).—The material of *S. americanus* and of *S. olneyi* collected at Hyannis grew by a small pool of water at the foot of Garnold Street, and the stations of the two species merged into each other. In this middle region there were found specimens of *S. americanus* which were taller than, and

different from, the other members of the species, and the suspicion was held that the two species had crossed.

Fig. 16 shows the great range in size of the chromosomes found in diakinesis. Beyond this, these stages are of little value in providing information.

In the metaphase plates there is always a large number of chromosomes. Fig. 17 shows 64 elements, but the chromosomes may range from 50 up to that number. The difference in size is striking; 12 of the chromosomes are larger. From a side view the chromosomes are all split in the anaphase of the heterotypic division. This statement of the fate of the smaller chromosomes, which must be interpreted as univalents, is borne out by inspection of the profile view. All elements in the late anaphase show a twofold nature.

While there is great irregularity in the manner by which the chromosomes go to the poles (fig. 18), no cases have been found in which the chromosomes are left in the cytoplasm.

The homotypic divisions appear to be regular and all the chromosomes are split. In one case there were found 5 nuclei in the pollen mother cell, although no exact basis for this formation has been found. To establish this condition a careful checking is necessary, as the nuclei from a neighboring cell may be included.

*Scirpus campestris* Britton var. *fernaldi* (Bicknell) Bartlett.—Material of this variety from a single station in Cape Breton Island was kindly provided by Professor JEFFREY. The pollen mother cells need the utmost care in staining and differentiating, for the cytoplasm often possesses a bluish cast, despite the effort to have it clear and still retain the desired color in the chromosomes. The chromosomes are very small and numerous. In diakinesis there is dark-staining material in the cytoplasm. When the spindle is formed there are three poles at first, but this soon gives way to the bipolar condition.

The chromosomes are arranged very irregularly on the spindle and are impossible to delimit, but one can distinguish larger and smaller elements. The scattered condition persists even in late metaphase (fig. 19).

Fig. 20 represents the most peculiar condition of the anaphase. No earlier stages have been seen, but in the late condition a few

chromosomes are found on the spindle, while more occupy the poles. Surrounding the whole chromatin mass is apparently a membrane, and around the periphery chromatin masses are arranged. In the upper corner of the figure it would appear as if some of the chromatin were being extruded into the cytoplasm. In interkinesis (fig. 21) the daughter nuclei have reorganized and possess nucleoli. Between the nuclei appear chromosomes that have been left out in the cytoplasm, and in the cytoplasm generally dark-staining material is present. The homotypic division has always been found to be regular, but the elements are always clumped together.

In other cases the spindles of the heterotypic division possess regular plates, and there is present a number of chromosomes which, in the majority of countings, is very close to 55. Even in these regular divisions there is also dark-staining material in the cytoplasm. In the nuclear abortion, the dark elements described in the cytoplasm are in part included.

The pollen is largely imperfect, but in contrast to this condition, the embryo sac mother cells develop normally, so that crossing with other plants is possible. The cells of the megaspore mother cells are very large and are usually cut by the knife. After the heterotypic division in these, there is the same chromatoid material in the cytoplasm.

*Scirpus campestris* var. *paludosus* (A. Nelson) Fern.—The material of this variety came from stations on the Charles River marshes at Watertown, and from Hyannis, Nahant, and West Manchester, Massachusetts.

The pollen mother cells are much more regular in this variety than in the Cape Breton material. The plants from West Manchester are transitional between the varieties *paludosus* and *novae angliae*, in that they tended to an umbellate condition; but the cytological conditions are the same as those found in other stations.

In all the plants from the various stations, the dark-staining material seems to increase as meiosis progresses, and the vacuole in the apex becomes partly filled with it. The majority of counts average around 55, but as high as 56 and 57 have been made. The pollen in these is good and bad.

*Scirpus fluviatilis* (Torr.) Gray.—A single collection of this species was made at Heard's Pond, Wayland, Massachusetts.

A metaphase plate of the heterotypic division (fig. 22) shows 55 chromosomes. This number represents an average of many counts. Counts vary, due to the fact that the chromosomes come to the equator quite irregularly.

All stages of division have been found to be regular, and the size of the pollen mother cells is the largest of all the species investigated. While the divisions are regular, an increasing amount of dark-staining material is found in the cytoplasm as meiosis progresses, and as usual this material is surrounded by a clear area. After the divisions are over, the 4 main nuclei present an appearance different from the other "nuclei."

*Scirpus robustus* Pursh.—Two stations of this species have been investigated, one at Oak Island and the other at Neponset River Reservation, Milton, Massachusetts.

Material from both regions presents a muddy appearance in the cytoplasm, which renders differentiation a matter of great painstaking. Counts made in the heterotypic metaphase (fig. 23) plates range from 53 to 54 and 55 in equal numbers. The gross behavior of the chromosomes indicates no irregularity, save for the presence of the usual dark-staining material in the cytoplasm.

Exotic material of the *Scirpus maritimus* group (identified as *S. robustus* by COCKAYNE) was collected by JEFFREY in New Zealand. Counts of the metaphase plates vary exceedingly, as may be gathered from the condition depicted in fig. 24.

*Scirpus caespitosus* L., *S. planifolius* Muhl., and *S. hallii* Gray have also been investigated, and while no statement can be made on the chromosome numbers, the conditions of sporogeny are in line with the other material.

### Discussion

The condition known as cytomixis is noticeable, very frequently, from the early stages of the prophase even up to the heterotypic metaphase. GREGORY (15) noticed it in sterile races of *Lathyrus odoratus*, and he describes his figures 16 and 17 as "the incomplete and abnormal division by constriction of the pollen mother cells, which occurs in a few cases." GATES (12) first coined this word for the



process of chromatin extrusion from the nucleus of one pollen mother cell into the cytoplasm of a neighboring one in *Oenothera gigas* and *O. biennis*, and it has been used ever since. He considered it a normal process, and apparently it causes no diminution in the chromosomes. ROSENBERG (34) records the occurrence in *Crepis* and *Drosera*, and ascribes it to faulty fixation. GATES and REES (13), working on pollen development in *Lactuca*, found this irregularity in all the species investigated, and stated that it was confined mostly to the early stages of the prophase, although there was one instance in the heterotypic anaphase. Miss YASUI (50), working on the cytology of some artificially produced hybrids, described in the prophase a passage of chromatin from one pollen mother cell through the pits of the membrane of the cell. SINOTO (40) made some observations on *Iris japonica* Thunb., and likewise deems cytomixis as due to poor fixation and mechanical injury. LONGLEY (32), writing of the dark material which he found in *Crataegus*, states: "I have seen what appeared to be an actual passing of this dark-staining through the walls between the pollen mother cell and tapetal cell."

In all the species of sedges wherein this odd occurrence is found in the prophase, the chromatin material makes its way from the nucleus out into the cytoplasm and passes over into the adjoining cell by spinning out into a fine thread. On entering the cytoplasm of a neighboring cell, the material rounds itself into a globular mass and creates a clear area around it. At times several such penetrating strands run across and coalesce. The result is not unlike a nucleus in appearance. When it occurs in diakinesis, the material passing over appears to be made up of chromosomes, the chromosome passing out into the cytoplasm and becoming a fine thread as it passes across. The same behavior appears where chromosomes pass over in the heterotypic metaphase.

The phenomenon is most difficult to explain, but it is to be observed in the present connection that the material was not handled roughly, and the best of care was taken in preservation. It is of further interest to note that the occurrence has taken place in almost all the common and standard fixatives: Flemming's strong solution, Bouin's, Benda's, Hermann's chromo-acetic, Carnoy's, Merkel's alcohol and acetic, both strong and medium.

Whether or not the dark-staining material appearing in the cytoplasm is in part the after effects of cytomixis cannot be decided. In the plants under consideration, the dark material where it occurs is first seen quite often in the middle and late stages of the prophase. In the narrow apices of the pollen mother cells, a vacuole is frequently found in radial aspect. Often in diakinesis these vacuoles are not filled with any material, but after the heterotypic division they become partly filled with dark material, staining the same as chromatin, and there is in addition more of the material to be found in the cytoplasm. After the homotypic there may be a further increase. Some of the material may be interpreted as being chromatin, as in the case of the mule.

The fate of the material has already been discussed. Some is included with the aborting nuclei, and the rest apparently disorganizes and disintegrates during the time that the exine and intine coats are laid down; for rarely, if at all, is there any of the material left in the pollen cell.

SHARP (39), in dealing with the term hybridization, is of the opinion that "the sexual union of any two protoplasts differing in hereditary potency, no matter how slight the difference, constitutes an act of hybridization in the fundamental sense." A survey of the literature on hybrid cytology shows that it is upon the degree of the difference of hereditary potency that the differences in behavior found in sporogeny depend.

This view is borne out by the results of GUYER (16), who crossed closely and more distantly related pigeons. He found that sterility increased in direct proportion as the parents were divergent from one another. The sterility was the result of abnormalities in meiosis; greater or less degeneration of the germ cells; and abnormalities in the structure of the spermatozoa, which showed great variation in size. He assigns the reason of sterility to the fact that there is lack of union in synapsis because of the incompatibility of the different plasmas.

SCHÜRHOFF (38) investigated a hybrid *Saxifraga* between *S. decipiens* and *S. granulata*. CORRENS had found the form occurring spontaneously, and later crossed the two parents to demonstrate the parentage of the hybrid. He found the meiotic divisions and the

pollen formation normal, and we must conclude that the parents were very closely related because of the manifested compatibility.

BLACKBURN and HARRISON (2) studied the two crosses *Salix viminalis* × *S. purpurea* and *S. caprea* × *S. lanata*. These two might have been taken as pure species, for there is regular pairing in all the different phases. The divisions are regular, and there is no slightest evidence of a hint at hybridity, for the pollen is good in addition. In the cross *S. aurita* × *S. phylicifolia* there were irregular heterotypic and homotypic divisions which resulted in small nuclei. Tetrads were always formed, however, but degenerated, resulting in sterile pollen.

In the case of the sterile hybrid *Ribes schneideri*, TISCHLER (45) shows in its mitosis no more irregularity than in *R. gordiana*, but the pollen mother cells are found in contrast to give rise to sterile gametes.

It is not without sufficient grounds, then, that the idea is put forth that we should not expect any great irregularities if hybridism occurs in some of the sedges investigated, especially when the species are so similar in the immature condition that the specific characters often cannot be distinguished. This last condition is particularly true of the huge genus *Carex*, investigated by HEILBORN. It is further a notable fact in many hybrid and apogamous plants that, while the pollen may become abortive, the megaspore mother cell is quite able to function and seeds may be developed; and since pollen formation in the sedges is analogous to the formation of the megaspore, and since there is only one chance in four to show any incompatibility, it may not be expected that any great irregularity manifests itself.

FOCKE (10) records *Syringa rothamogensis*, a cross between *S. vulgaris* × *S. persica*, on the basis of its completely sterile pollen and of its history as it arose under garden conditions. JUEL (26) sought the causes of sterility and found them in the abnormalities of tetrad formation. The chromosomes from the parents seemed to be incompatible in part, and the irregularities followed as a consequence. TISCHLER (45) examined the same form cytologically, and reported regular as well as irregular meiosis. He believes that other factors as well as hybridity can be the cause of irregularity. More recently,

Miss BORGSTAM (3) has sought to demonstrate that lowering of the temperature causes the conditions as found by JUEL and TISCHLER.

A serious objection to Miss BORGSTAM's interpretation is that the form is of known hybrid origin, and more reliability could be placed on her results had a known homozygous form been chosen. Many hybrids have been described which have both irregular and regular divisions, and further hybrids often show more irregularity in the first generations than in those that follow.

It would seem that *Scirpus acutus* f. *congestus* is most probably of heterozygous origin, because of the condition of the microspores and on the basis of its regional variability. This view has long been held by JEFFREY (22, 23, 25) and by his students (FORSATH 11, COLE 5, LONGLEY 31, 32).

The roses have also been distinguished by their polymorphy, and the cause was unknown or only suspected for some time. The work of TÄCKHOLM (42, 43) provides almost an epitome of our knowledge of hybrid cytology. He divides this great group into three great classes: (1) those characterized by the occurrence of only paired chromosomes; (2) the very polymorphic *Canina* section in which bivalents and univalents are found, usually in multiples of seven; (3) aneuploid forms in which bivalents and univalents do not form multiples of seven.

Many of the recorded hybrids of the first class are regular in their reproductive divisions and the formation of spores. The *Canina* groups are of special interest because they are analogous to the well known *Drosera* scheme of hybrids. By their crossing, the *Canina* groups have given rise to the aneuploid species. The numbers here do not form multiples, due to the fact that the pollen grains do not possess the full quota of chromosomes, because of the double splitting of the univalents as found in the irregular forms of *Scirpus americanus*, and because of the irregularities in distribution during division. These aneuploid numbers, due to the agency of hybridism, are of great importance in connection with the explanation of the origin of dysploidy in other plants. It will be seen that the roses are no exception in this regard.

GEERTS (14) crossed *O. lamarckiana* × *O. gigas*, and found as a result that there were 7 bivalents and 7 univalents. Chromosomes from the gemini go to form the main tetrad nuclei, while the univalent chromosomes go to form mainly dwarf nuclei. In the  $F_2$  generation he found 14 chromosomes, pointing to the elimination of the chromosomes which failed to pair in diakinesis. MISS LUTZ (29) in the first generations of the cross *O. lata* × *O. gigas* found the following numbers: 15, 21, 22, 23, 29, and 30. KIHARA (27) has observed in the  $F_2$ ,  $F_3$ , and  $F_4$  plants of pentaploid *Triticum* hybrids the numbers 28, 31, 32, 37, 38, 39, 40, 41, and 42. This case is especially interesting, as the  $F_1$  had 35. DE MOL (33) has found in cultivated races of *Hyacinthus* the following varying numbers: 19, 20, 21, 22, 23, 24, 27, 28, and 30.

TISCHLER (44) has studied a sterile *Bryonia* hybrid. In the prophase chromatin was to be found in the cytoplasm. Further, the chromosomes were arranged irregularly on the spindle and seldom form a regular plate. Likewise, the homotypic spindles were irregular, but supernumerary pollen grains were seldom found. The chromosomes not only lag but are also extruded into the cytoplasm. Later (48) he interpreted his findings as being due to crossing of species of unequal chromosome numbers.

In *Rubus* and *Crataegus* LONGLEY (31, 32) has found conditions which seem to explain polymorphy. He concludes that species have been modified by hybridization. In *Crataegus* he finds three classes: (1) diploid species in which pollen formation is normal; (2) triploid and tetraploid species that show irregularities in their chromosome distribution and are accompanied by polycary and polyspory; (3) triploid and tetraploid species that are unable to form pollen grains and are sterile, due to the fact that they are the products of distant crosses. He divides *Rubus* into two major classes: (1) diploid species in which meiosis and pollen formation are normal; (2) polyploid species which are triploid, tetraploid, pentaploid, hexaploid, and octoploid. These polyploid forms are characterized by irregularities in chromosome distribution which lead to polycary and polyspory.

Wheat has been the subject of much investigation. SAX (36, 37) divides the species on the basis of the gametophytic number: Ein-

korn 7, Emmer 14, and Vulgare 21. Crosses between the different sorts give rise to cytological behavior like that in *Drosera obovata*. He finds that sterility increases in proportion as the number of univalent chromosomes increases in the reduction divisions. The figures of the anaphase are not unlike those of the irregular variety of *Scirpus campestris* found in Cape Breton.

FARMER and DIGBY (6) investigated *Polypodium schneideri*, which arose as a cross between *P. aureum* and *P. vulgare* var. *elegantissimum*. The spores were incapable of germination. The haploid number in *P. aureum* is 34, while the corresponding number in the other parent is 90. The hybrid, however, possessed usually from 95 to 105 chromosomes, seen in the late prophase, and in a few cases more were found: an indication that the number of chromosomes which pair is by no means a fixed one. The mitoses are very irregular, and often regression changes develop in the spore mother cells, with the result that the tetrads are but rarely formed and soon degenerate.

WODSEDALEK (49) has investigated the causes of sterility in the mule. It is in many respects the zoological counterpart of the hybrid fern. The divisions in the spermatogonial cells are quite normal, but in the late prophase of the primary spermatocyte the pairing of the chromosomes is never complete. It is also inconsistent, as seen in the number of chromosomes present, which ranges from 34 to 49 as compared with the 51 chromosomes found in the spermatogonial cells. The abnormalities invariably occur in the metaphase of the primary spermatocytes, and there is evidence that the chromatin material is eliminated. The result is almost complete sterility. The work provides an absolute demonstration of the concomitancy of hybridity and sterility.

The Boston fern has long been known for its extreme variability, almost complete sterility, and great vegetative vigor. The causes of polymorphism in this plant have been described by JEFFREY and ROSCOE (25). The reduction divisions are quite abnormal, the sporangia are shrunken, and the spores are abortive in their development since they are shriveled and devoid of protoplasmic content. Accordingly, this so-called mutating plant is apparently to be regarded as a hybrid.

### Conclusions

The evidence presented has emphasized the point of view that, not only has polyploidy originated through crossing, but aneuploidy as well finds its explanation in that phenomenon. The conditions that have been described, in a genus that is comparatively stable taxonomically, have provided significant suggestions toward explanation of non-multiploid polyploidy in the American species of *Scirpus* and in other groups, namely, as the result of crossing.

*Scirpus campestris* var. *fernaldi* shows abnormal meiotic divisions, and the definite cases of chromatin extrusion seen in the pollen mother cells seem to indicate that this form has arisen through crossing. Material from other stations of this species in Massachusetts shows fairly regular divisions but some sterility. The dark-staining material present in these forms, as well as in other species, leads to the suspicion that this chromatoid material may be further evidence of crossing.

Other definite cases where hybrids have apparently arisen are to be found in *Scirpus acutus* f. *congestus*, because of the manifested sterility and loose affinity of the chromosomes in meiosis. Further, the cytological irregularities of *S. americanus* at Hyannis, manifesting the failure of chromosomes to pair, seem to be clear indications of heterozygosis. The cytological conditions found here may also serve to explain the cause of great variation in size, shown by more regular forms of this species in different localities.

In view of the cytological evidence of hybridism here presented, it is to be concluded that *Scirpus* is apparently being modified by crossing, and it is highly probable that the same situation has also prevailed in the past.

An interesting question arises in regard to the conclusions reached by HEILBORN in *Carex*. As has been pointed out in this paper, HEILBORN comes to the conclusion that the non-multiploid (dysploid) polyploidy found in *Carex* is the result of mutation, and has no obvious connection with previous hybridism. It seems unlikely, in view of the conditions here recorded for *Scirpus*, that this point of view can be maintained.

## Summary

1. Aneuploidy is present in American species of *Scirpus*.
2. The chromosome numbers found range as follows: 18, 20, 21, 25-30, 28, 33, 34, 38, 39, 50-64, 53-55, 55, 55-57.
3. Conditions similar to that found in known hybrids have been discovered.
4. The seemingly hybrid conditions are to be correlated with taxonomic variability and polymorphy.
5. The interpretations of HEILBORN cannot be applied to the species investigated.
6. Hybridization is offered as a probable explanation of the aneuploidy found.

The writer wishes to record here his indebtedness to Professor E. C. JEFFREY, at whose suggestion this research was undertaken. Appreciation is also expressed for the plant determination checks made by Professor M. L. FERNALD.

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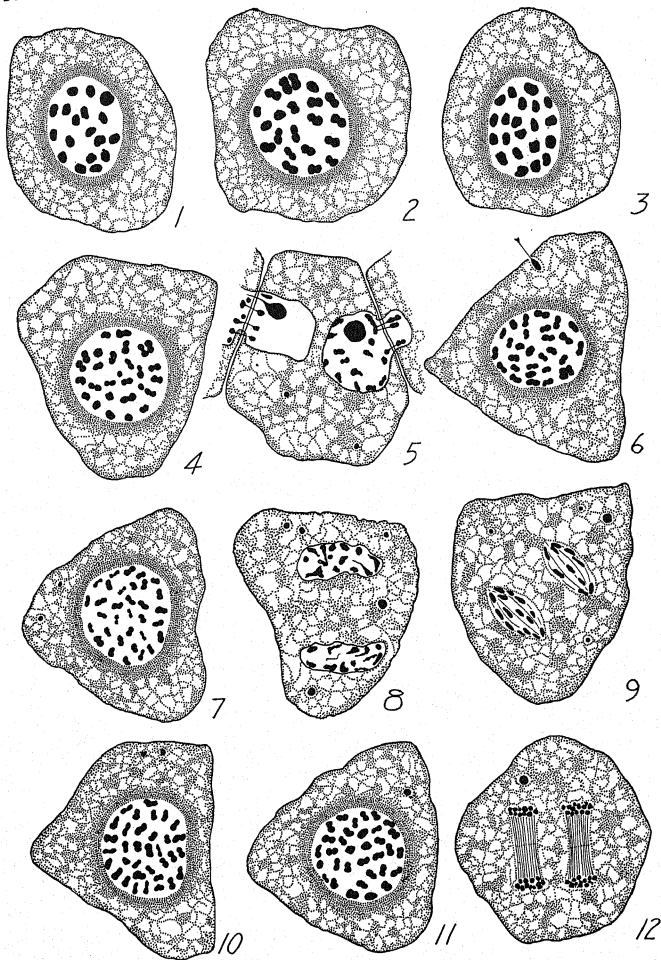
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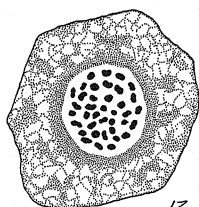


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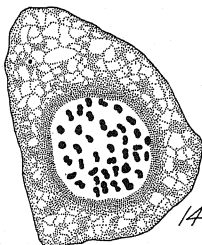
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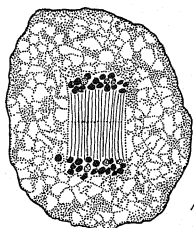




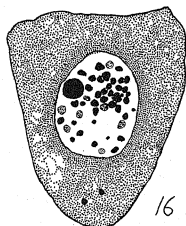
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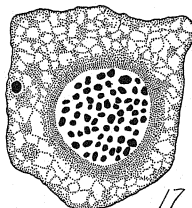
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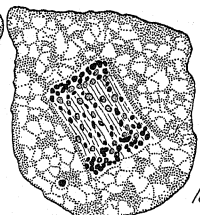
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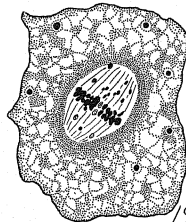
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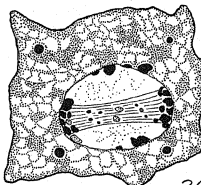
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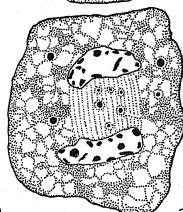
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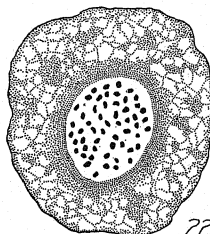
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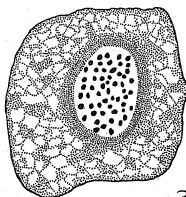
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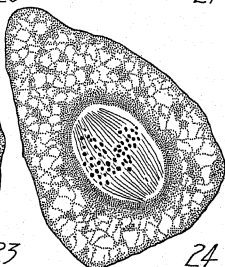
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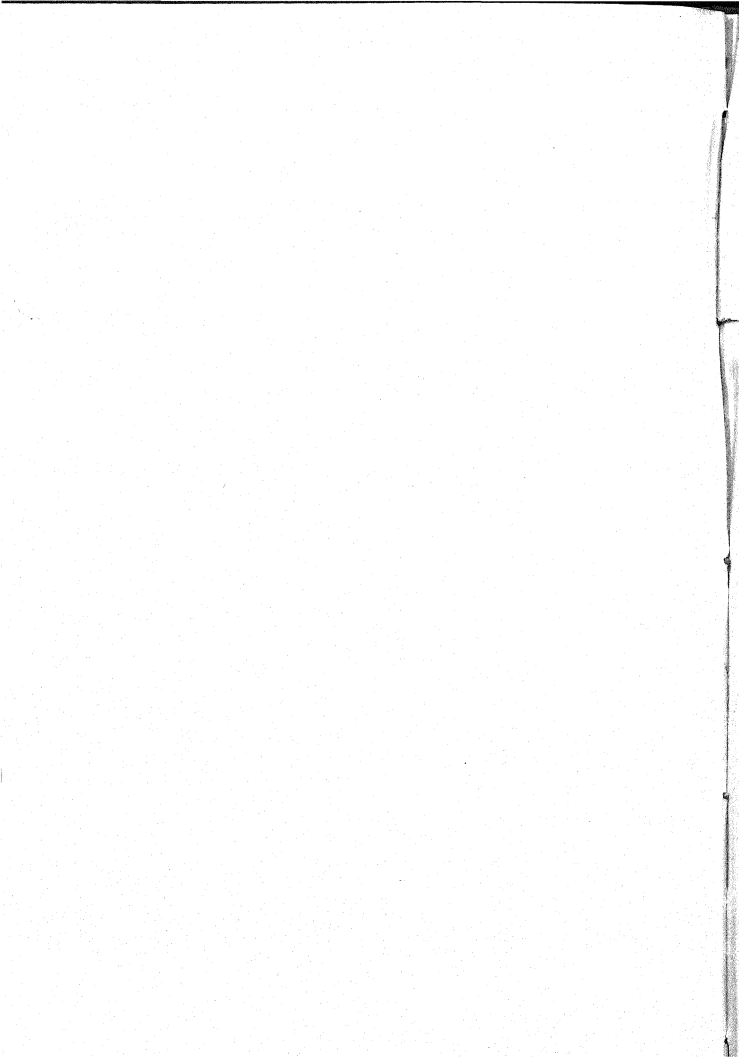
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## EXPLANATION OF PLATES X, XI

## PLATE X

- FIG. 1.—*Scirpus heterochaetus*: metaphase of first division, polar view.  
FIG. 2.—*S. acutus* f. *congestus*: metaphase of first division, polar view.  
FIG. 3.—*S. validus*: metaphase of first division, polar view.  
FIG. 4.—*S. atrovirens*: transverse section, polar view of metaphase of first division.  
FIG. 5.—Same: cytomixis.  
FIG. 6.—*S. atrovirens* var. *georgianus*: polar view of metaphase of first division.  
FIG. 7.—*S. rubroinctus*: transverse section, polar view of metaphase plate.  
FIG. 8.—*S. longii*: anaphase of first division, transverse section.  
FIG. 9.—Same: early metaphase of second division.  
FIG. 10.—*S. atrovirens*: transverse section, polar view of metaphase plate.  
FIG. 11.—*S. cyperinus* var. *pelius*: metaphase of first division, polar view.  
FIG. 12.—Same: anaphase of second division showing cell plates.

## PLATE XI

- FIG. 13.—*S. americanus*: metaphase of first division, polar view, tangential section.  
FIG. 14.—*S. olneyi*: metaphase of first division, polar view.  
FIG. 15.—Same: anaphase of first division, tangential section.  
FIG. 16.—*S. americanus*: irregular form, diakinesis.  
FIG. 17.—Same: metaphase of first division, polar view.  
FIG. 18.—Same: anaphase of first division, transverse section.  
FIG. 19.—*S. campestris* var. *fernaldi*: diakinesis.  
FIG. 20.—Same: anaphase of first division.  
FIG. 21.—Same: telophase of first division.  
FIG. 22.—*S. fluviatilis*: metaphase of first division.  
FIG. 23.—*S. robustus*: metaphase of first division.  
FIG. 24.—Same: New Zealand form, metaphase of first division.

## ROOT INHERITANCE IN PEAS

F. C. JEAN

(WITH FOUR FIGURES)

### Introduction

Since the time of MENDEL (5) it has been known that above-ground characters, such as tallness and dwarfness in peas, are transmissible from parent to offspring. Little or nothing, however, is known about the hereditary behavior of the roots. A review of the literature reveals not only that no previously recorded investigations as to the hereditary relations of pea roots have been made, but that the genetic behavior of roots in general is almost a wholly unexplored field.

HOWARD and HOWARD (4), investigating the fiber plant *Hibiscus sabdariffa*, found the tendency for the roots to discolor in wet seasons and an intolerance of constantly wet soil to be inherited. HOLBERT and KOEHLER (3), while investigating the extent and anchorage of corn root systems, discovered an apparent correlation between certain root characters and a susceptibility to leaf firing and root rot. Their data "suggest that the genetic factors responsible for the reduced root systems in the strains susceptible to root rot and leaf firing, respectively, are recessive." COTTLE (1) found the tendency to lodging in certain strains of corn to be due to a fragility of roots which was transmitted. VENKATRAMAN (7), making a study of sugar cane, felt that his observations indicated the inheritance of, and influence of the pollinating parent on "depth, penetration, resistance to water logging, and other characters of the root."

These statements are found, with the exception of VENKATRAMAN's study, in the reports of research not primarily concerned with root inheritance. They are incidental only, and tend to show just how little definite work has been done in this field. This investigation was carried on in an attempt to determine whether root length is a genetic factor in peas, and whether it segregates in the  $F_2$  generation as does the height of the top.



### Material and method

Commercial varieties of Nott's Excelsior and of Telephone peas, both strains of *Pisum sativum*, were selected for this work. The first is a dwarf plant which, under field conditions, attains a height of about 1 ft. 8 in.; the latter reaches a height of approximately 4 ft., or more than twice that of the small plant.

In excavating the root systems, the trench method, as employed by WEAVER, JEAN, and CRIST (8), was used with slight modifications. This consisted in digging a trench of convenient size, about a foot from the base of the plants, to a depth well below the maximum

TABLE I  
VALUES OF TOP AND ROOT MEASUREMENTS AND THEIR RELATIONS  
(TOP MEASUREMENT FOR 20 PLANTS, ROOT MEASUREMENT  
FOR 10 PLANTS)

PLANTS	RANGE OF HEIGHT (IN.)	MEAN HEIGHT (IN.)	PERCENT- AGE RELATION OF HEIGHT	RANGE OF MAXIMUM ROOT PENE- TRATION (IN.)	MEAN MAXIMUM ROOT PENE- TRATION (IN.)	PERCENT- AGE RELATION OF ROOT PENE- TRATION
Parental Nott's Ex- celsior.....	13 to 27	19.2	100.0	22 to 34	28.2	100.0
Parental Telephone.....	36 to 54	46.5	242.2*	28 to 45	35.8	127.0
F <sub>1</sub> generation.....	36 to 48	41.1	214.1	31 to 49	37.9	134.4
Dwarf F <sub>2</sub> segregates.....	14 to 26	20.0	104.2	25 to 37	30.9	109.6
Tall F <sub>2</sub> segregates...	38 to 56	44.9	233.9	30 to 50	40.9	145.0

\* In computing the percentage relations for the mean height of tops and the mean depth of roots, the values for the parental Nott's Excelsior were taken as 100 per cent.

root penetration. The roots were then dissected out of the soil with appropriate tools, measured, and sketched to scale. The root systems of ten plants in each parental and hybrid generation were examined.

### CULTURE AND ENVIRONMENTAL CONDITIONS

The original parents were grown in the greenhouse at the University of Nebraska during the winter of 1924-25. Stamen emasculation was made under a hand lens to insure that no self pollination occurred. Cross pollination was then accomplished by hand in the usual manner, and the flowers immediately bagged to prevent possible insect contamination. The F<sub>1</sub> generation was grown during the season of 1925 in a field near Greeley, and permitted to self fertilize.

In 1926 representatives of all generations, that is, both parental

lines and the  $F_1$  and  $F_2$  generations, were grown for the purpose of examination and comparison. The seeds were planted April 26 in separate, adjacent plots. The rows were 3 ft. apart, and the plants were spaced at intervals of 3 in. in the rows. Surface cultivation was given from time to time, to prevent the growth of weeds and to keep the soil in good tilth.

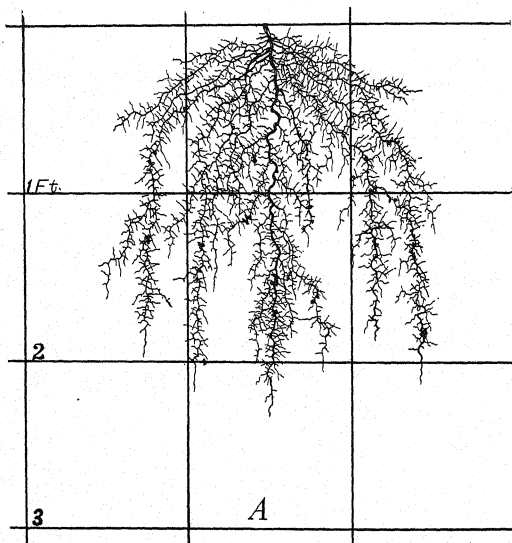


FIG. 1 A.—Dwarf pea root system: Nott's Excelsior, parental

The weather was characteristic of northern Colorado east of the Rockies. The precipitation from May 1 to August 1 was 5.7 inches, most of which came in light showers. The mean night temperature for the same period was 59° F. and the mean day temperature 76° F. The average night and day humidities were 89 and 48 per cent

respectively. Soil moisture determinations were made regularly, and when, on June 7 and 28, the soil became too dry for good growth the plots were irrigated.

#### TOP AND ROOT CHARACTERISTICS

All plants were examined July 9 and 10. The parental Nott's Excelsior were practically mature. Some blossoms were still to be

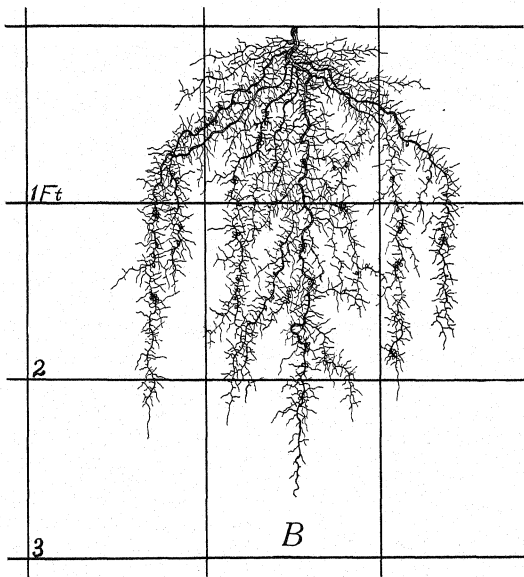


FIG. 1 *B*.—Dwarf pea root system:  $F_2$  segregates

seen, but many of the fruit pods were so old as to be light colored and crinkled. The average height of twenty successive plants was 19.2 in. (table I).

The root system was characterized by a tap root which pursued a more or less vertical course downward through the soil. In three of the ten plants examined the tap root had died at 3-8 in. below the surface, its functions then being assumed by one or more of the larger laterals. The maximum depth of penetration ranged

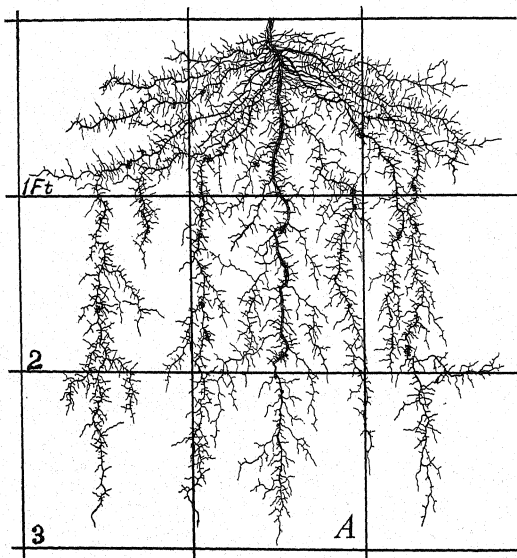


FIG. 2 A.—Tall pea root system: Telephone, parental

from 22 to 34 in., the average being 28.2 in. (table I and fig. 1 A). At 4-6 in. beneath the surface many large laterals took their origin from the tap root. Some of these ran off obliquely to a distance of 6-13 in., terminating at a depth of 5-8 in. below the surface. Others ran obliquely 6-9 in. from the vertical and then turned downward,

pursuing a course almost parallel with the tap and penetrating to a depth of 12-28 in. Many reached a depth of 24 in. The tap root as well as all of the main laterals was well supplied with branches

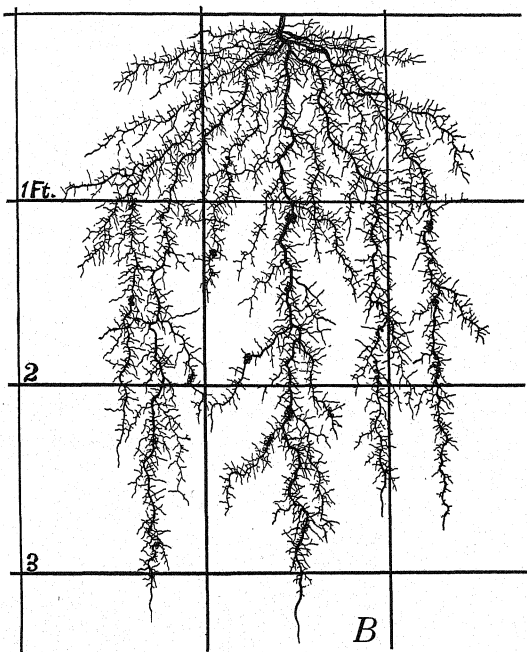


FIG. 2 B.—Tall pea root system:  $F_2$  segregates

0.1-4 in. in length. There was an average of seven branches per linear inch. Some tertiary branches also occurred. The roots bore nodules rather sparsely to a depth of approximately 2 ft.

The parental Telephone peas, as compared with the Nott's Ex-

celsior, were slightly less mature when examined; however, some of the pods were white and crinkled, and little or no further growth occurred. An average of twenty plants gave a height of 46.5 in. (table I). The root system was similar in form to the smaller pea,

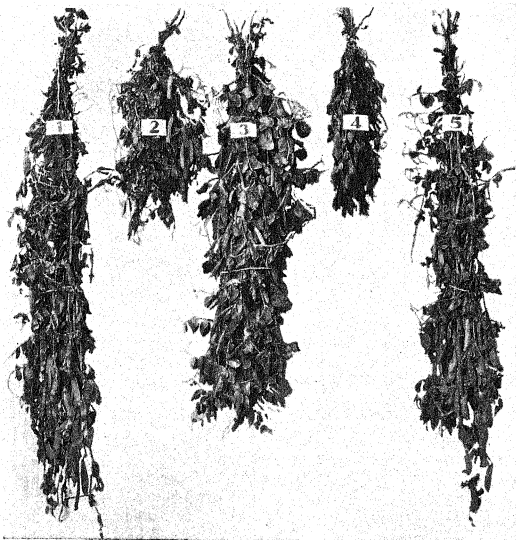


FIG. 3.—1, Parental Telephones; 2, parental Nott's Excelsior; 3,  $F_1$  hybrid; 4, dwarf  $F_2$  segregates; 5, tall  $F_2$  segregates.

but all of its dimensions were greater (fig. 2 A). The maximum depth of penetration ranged from 28 to 45 in., with an average of 35.8 in. The smaller secondary branches were abundant, there being eleven per inch as compared with seven for the Nott's Excelsior. Nodules were also more abundant to a depth of over 2 ft.

The  $F_1$  plants were in approximately the same stage of develop-

ment as were the parental Telephones. They were slightly shorter, but in other respects their above-ground characters were strikingly like those of the dominant parent. An average of twenty plants gave a height of 41.1 in. (table I). The root system was almost a counterpart of the larger pea in regard to form and branching. Its maximum penetration was a little greater, however, although its height of top was slightly less.

The  $F_2$  generation of hybrids showed an almost exact Mendelian ratio in respect to height. Of the 277 plants that reached maturity, 209 were tall and 68 were dwarf (fig. 3). This gave a ratio of 3.07:1.

The dwarf  $F_2$  segregates ranged in height from 14 to 26 in., with an average of 20 in. The tall  $F_2$  segregates varied from 38 to 56 in., with a mean of 44.9 in. (table I). The smaller segregates had a root system almost identical with that of the recessive parent, except that its maximum penetration was slightly greater (fig. 1 *B*). These dwarf  $F_2$ 's also bore nodules more profusely than did their similar parent, which may have been due either to some character inherited from the dominant line, or to their close proximity to the larger segregates, which, like their corresponding ancestor, produced tubercles to a marked degree. On the other hand, the tall segregate strongly resembled the dominant parent in respect to underground parts; indeed, the two root systems could scarcely be distinguished, except that the  $F_2$  individuals penetrated the soil a little deeper. The maximum penetration of ten plants varied from 30 to 50 in., with an average of 40.9 in. (fig. 2 *B*).

### Discussion

The data are limited to ten individual root systems in each case. The number was necessarily so restricted because of the time, labor, and expense involved. Since in the  $F_2$  generation individual segregates were examined, it was frequently necessary to excavate a trench in order just to secure the measurements on a single root system. Conclusions drawn on the basis of this limited number of plants must be considered indicative and tentative only. Final conclusions must await further investigation.

An examination of the data suggests strongly the lack of any agreement between the ratios of root penetration to height of top

in the case of the two varieties of peas studied. For instance, the mean maximum root penetration and height for Nott's Excelsior was 28.2 and 19.2 in. respectively. This gives a ratio of 1.4. The Telephones, on the other hand, had a mean maximum root penetration of 35.8 in. and a mean height of 46.5 in. This gives a ratio of 0.7, which is only one-half as great as in the case of the small pea. The reasons for this lack of correlation may be due to several causes working either separately or in combination. It might result from a difference in the transpiring power of the tops, from a difference in the leaf areas, from a difference in the absorbing power of the roots, or from a difference in the branching habits of the roots which would affect the absorbing area. That this disparity between the root and top ratios is not a fortuitous one, however, is strongly supported by the  $F_2$  segregates. Here the ratios are 1.5 and 0.9 respectively, which is almost the same relation as was found in the case of the parents.

Although the difference in root length between the two parental varieties is not great, the data show a strong tendency for such difference as there is to segregate along with height. In table I the range in height for Nott's Excelsior is found to be 14 in. with a mean of 19.2 in., and the variation in root length 12 in. with a mean of 28.2 in. The smaller  $F_2$  segregates show a height range of 12 in. and a mean of 20 in., with a variation in root length of 12 in. and a mean of 30.9 in. Likewise, the parental Telephones show a height range of 18 in. with a mean of 46.5 in., and a root length variation of 17 in. with a mean of 35.8 in. The larger segregates have a height range of 18 in. with a mean of 44.9 in., and a root length variation of 20 in. with a mean of 40.9 in.

This close agreement in respect to the relation existing between height and root length, as between the parental lines and their  $F_2$  segregates, might be due to a response to the transpirational demands of the plants, the taller tops requiring the longer roots. To decide this point the tops of ten parental Telephone plants were clipped off when they had reached a height of 9-10 in., and were kept pruned back to this height during the period in which the other plants were developing. The side shoots and flower buds that started on these pruned plants were pinched off as fast as they appeared, thus retaining as nearly as possible the original reduced area of



stem and leaves. Fig. 4 is a representation of the root system developed by these pruned plants. The measurements are given in table II. It may be seen by a comparison of fig. 4, fig. 2 *A*, and table II that, although the height of the clipped plants was only about

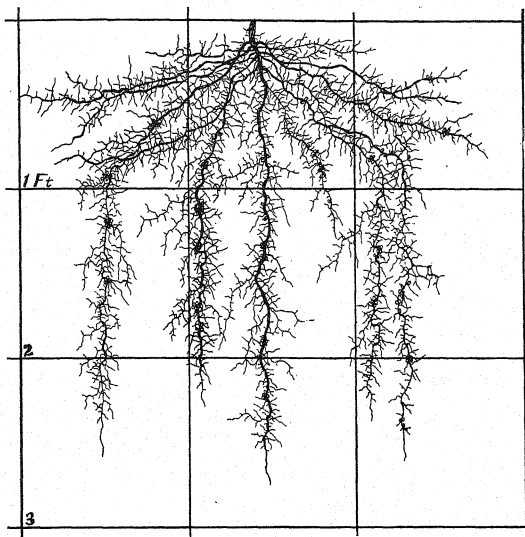


FIG. 4.—Root system of pruned Telephone plants; season 1926

one-fifth as great as those with normal tops, the root penetration was approximately within 2 in. as deep. Since these plants were legumes, taking part of their nitrogen supply from the air through their symbiotic bacteria, and also since the plot had been well fertilized, nitrogen hunger could have had little influence upon root growth (GERICKE 2, REID 6). The evidence, then, suggests strongly that hereditary genes accompanying those that determine height are the causative factors that determine root penetration in the

case of peas, and not the physiological demands alone of the above-ground parts.

The fact that pruned Telephone peas, in the summer of 1926, produced roots practically as long as those unpruned was unexpected, and it was decided to check this relation again in 1927. Accordingly, several hundred seeds were planted early in the spring, but these like a second planting failed to germinate. Twelve plants were finally secured. Eight of these were clipped at 11 in. and kept at this height; the other four were permitted to grow.

At the time of excavation the plants had reached approximately the same stage of maturity as those examined the previous year.

TABLE II  
TELEPHONE PEAS

YEAR	PLANTS	NO. OF PLANTS	RANGE OF HEIGHT (IN.)	MEAN HEIGHT (IN.)	PERCENTAGE RELATION OF TOP HEIGHTS	RANGE OF MAXIMUM ROOT PENETRATION (IN.)	MEAN MAXIMUM ROOT PENETRATION (IN.)	PERCENTAGE RELATIONS OF ROOT PENETRATION (IN.)
1926 .....	Unclipped	10	36 to 54	47.0	244.8	28 to 45	35.8	127.0
1926 .....	Clipped	10	9 to 10	9.3	48.4	29 to 37	33.3	118.1
1927 .....	Unclipped	4	36 to 41	38.8	202.1	34 to 49	43.3	153.5
1927 .....	Clipped	8	0	11.0	57.3	34 to 47	39.5	140.1

Table II shows the top and root relations of both the clipped and the unclipped plants. The unpruned peas did not grow quite as tall as those of the previous year, but the roots of both the unclipped and the clipped ones penetrated more deeply. This was due to differences in the plots, which were a short distance from those of the previous year, the subsoil being less compact. The percentage relation of root penetration between the two lots, however, was very similar to that previously determined. This again indicates that root penetration in peas is determined by genetic factors rather than by the extent of the leaf area.

Had they been available, it would have been desirable in this investigation to use pure parental lines selected for uniformity of height. But, since peas are normally closely selfed, it is doubtful whether this condition would have changed the results appreciably. In so far as an examination of ten plants of each kind is significant, therefore, this work indicates that root length in peas, as well as height of tops, segregates in the  $F_2$  generation; moreover, that these

top and root dimensions are linked in heredity. The data and figures also show that the root forms in the varieties studied are very similar, and that such differences as do exist are largely ones of magnitude.

### Summary

1. There exists a great varietal difference in the ratio relation of root and top length between Nott's Excelsior and Telephone peas.
2. In the  $F_2$  generation height of top and root length seem to segregate in almost the same relation that these characters existed in the parental stocks.
3. Failure to change the root penetration significantly in the tall parent by pruning the plants suggests genetic factors as the primary cause of root length.
4. In the two varieties studied the forms of the root systems are very similar, such difference as does exist being largely one of dimensions.

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## CYTOLOGY OF HALIDRYS DIOICA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 386

DOROTHEA G. DOUBT

(WITH SEVENTEEN FIGURES)

### Introduction

The brown alga *Halidrys dioica* is a species of the Fucaceae which was described by GARDNER (5) in 1913, and is known only from the coast of southern California. Its range extends from Redondo to a short distance beyond San Diego, and includes Catalina Island. No study has been made of the species aside from the identification of a fungus and two brown algae that parasitize it (4, 17).

With the exception of the species *H. dioica*, *Halidrys* is confined to the coasts of northern Europe and the British Isles (6). DE TONI (20) reported its existence in the Japan and China seas, but the report has not been confirmed by recent workers (22). GARDNER distinguished *H. dioica* from the European species by four characters: smaller, wider-winged, more lanceolate, shorter stiped air vesicles; more profusely branched fruiting ramuli which frequently arise from the side of the air vesicles; more "zigzag" character of the stipe and lower branches; dioecious habit.

Described in 1819 by LYNGBYE (11) of Denmark, *H. siliquosa* was worked successively by GREVILLE (7), AGARDH (1), and THURET (19) during the first sixty years with comparatively small results, excepting the contribution of AGARDH to the knowledge of air vesicles; but the studies made since that time by OLTMANNS (13, 14), BOWER (2), REINKE (16), FARMER and WILLIAMS (5), LE TOUZE (10), and NIENBURG (12) have been fairly complete. OLTMANNS, who has assembled most of these data, notes that the growth of the plant is initiated by a 4-sided apical cell, that there is bilateral branching, that a midrib is gradually differentiated from the isodiametric cells, that a considerable development of hyphae takes place in the inner region, and that air vesicles develop about the midrib.

WILLE (21) and HICK (9) had worked on some Fucaceae and been successful in finding protoplasmic connections, but HANSTEEN (8) was not certain that the protoplasmic strands were carried through the pores he observed, and LE TOUZE thought there was no protoplasmic connection. LE TOUZE alone worked on *Halidrys*.

The products of assimilation have been studied repeatedly in *H. siliquosa* and in a number of other Fucaceae. CZAPEK (3) thinks the small, highly refractive globules which are very widespread in cells of the Fucaceae are tannin vacuoles, and are identical with the fucosan which HANSTEEN first thought was a carbohydrate and later a glucoside. CZAPEK refers to KYLIN's work in which it was concluded that these bodies contain a phenol-like substance which is oxidizable, and which thus produces, after death, the coloring matter which has long since played so large a rôle in the chemistry of algae as phycophaein. CZAPEK credits MOLISCH and TSWETT with having discovered that it was a post-mortem product. Since CZAPEK's work, PALMER (15) has shown that the material oxidized after death to produce the pigment phycophaein is a colorless chromogen in living cells, while the color of living tissue is due to a carotinoid, fucoxanthin. PALMER indicates that chlorophyll is also present in these cells.

BOWER (2), OLTMANNS (13), and NIENBURG (12) have worked out the development of the conceptacle in *H. siliquosa*. BOWER reported that an initial cell slightly sunken in the epidermis cuts off two or more segments by horizontal divisions. Then the basal cell divides, the first two walls being more or less vertical but inclined toward each other. It is some time before these cells divide in a plane parallel to the surface of the cavity. As a result, the conceptacle appears to be lined by a layer of cells continuous with the limiting layer, but as part at least of this tissue was derived from the basal cell, BOWER thought that this conclusion was not correct. Meanwhile the initial cell (or group of cells) has been completely thrown off by the swelling of the wall dividing it from the basal cell. Later, as in other Fucaceae, the cells of the lining tissue put forth papillae which develop further into hairs.

OLTMANNS' series runs very much like that of BOWER. An epidermal initial, more or less rectangular in shape, cuts off a basal

segment and becomes slightly sunken below the level of its neighbors. These lateral cells initiate the wall of the young conceptacle, but its growth is mainly due to divisions of the basal segment of the initial cells. The upper segment of the initial has meanwhile cut off several segments by horizontal division, forming a hair, as described by BOWER. OLTMANNS does not say that the hair is thrown off, however; he believes rather that when the conceptacle has reached its full size the hair is no longer distinguishable from the paraphyses; and while he is uncertain of the final disposition of the hair, it does not contribute anything significant to the development of the conceptacle.

NIENBURG made his report in 1913, and was influenced strongly in his study by the work of SIMONS on *Sargassum* (18). He found the conceptacle initial of *H. siliquosa* very similar to that of *Sargassum*, only more vacuolated, shorter, and the tongue narrower. As in *Sargassum*, he found that the first division of the tongue cell cuts off an inclosing lower cell around the base of the upper one; the second division cuts the basal cell in half longitudinally; the third cuts each of these cells in half obliquely toward the center of the initial; and the fourth divides the upper cell on each side of the initial. Further development is as outlined by OLTMANNS.

#### Materials and methods

The material used in this study came from San Diego, California. A number of plants were collected there in September, 1927, and fixed in a solution of formalin, acetic acid, and sea water.

A preliminary study was made of freehand sections, and later paraffin sections were used.

In preparing the material for slides, it was necessary to remove the air from the vesicles before imbedding. This was done when they were in the xylol, by piercing each chamber with a fine needle and exhausting the air from the inclosing flask by means of a force pump. Parawax melting at 52° C. was used as an imbedding medium, and sections were cut at 3 and 5  $\mu$  for most of the work; while a few topographical sections were cut at 10  $\mu$ . With the longitudinal sections, best results were gained when the material was cut parallel to the narrow surface.

Several stains were tested out, singly or in combination, but Haidenhain's haematoxylin and orange G were best for detailed work in this species. The quantities of granular and globular cell contents, which added not a little to the difficulty of effective staining, made little or no trouble when treated in this way. For topographical work the safranin and haematoxylin combination was good.

### Investigation

The material used in the present study consisted of mature plants 75–85 cm. long. The male plants seemed to have considerably more abundant fruiting ramuli than the female, giving by comparison a very tufted appearance. It would be necessary, however, to examine a great many more specimens than were at hand to establish this character.

Air chambers were found developing in the leaves, in shoots that were elongated leaves, as separate air vesicles, and as portions of receptacle shoots (figs. 1, 2). Every possible intergrade was found between leaves and air vesicles. A leaf would give rise to another leaf or series of leaves, or to an air vesicle, or to both. There might be a single large chamber, or as many as three or more of them in the center of a very broad, flat leaf. Air vesicles of twelve chambers were frequently found, although eight or ten were most common. Groups of from one to fifteen chambers were noted in the portions of the shoots lying just under the receptacles. The shoots were uniformly green-brown in color, excepting the apices, which showed a lighter yellow tinge.

The assimilative and storage regions were examined, and found to contain mainly plastids of varying sizes and with colors from brown to green. The smaller plastids ranged down to the limit of vision, and were seen in stages of division. Especially large brown plastids averaging 7–8  $\mu$  in diameter were comparatively infrequent in the peripheral layer, but usually occurred in such quantities in the first two or three cells under it as to fill them to capacity, so that the nucleus was only occasionally visible. These plastids, called fuco-san, have a very dense center, which is highly refractive when small and may appear cracked. They also have a more or less lamellated structure such as potato starch plastids have, although they do not

respond to a test for starch. They may be single, double, or compound (fig. 3). Occasionally the contents of the plastids are dissolved out, leaving only the honeycomb network of the plastid walls, which resists disintegration for a longer time.

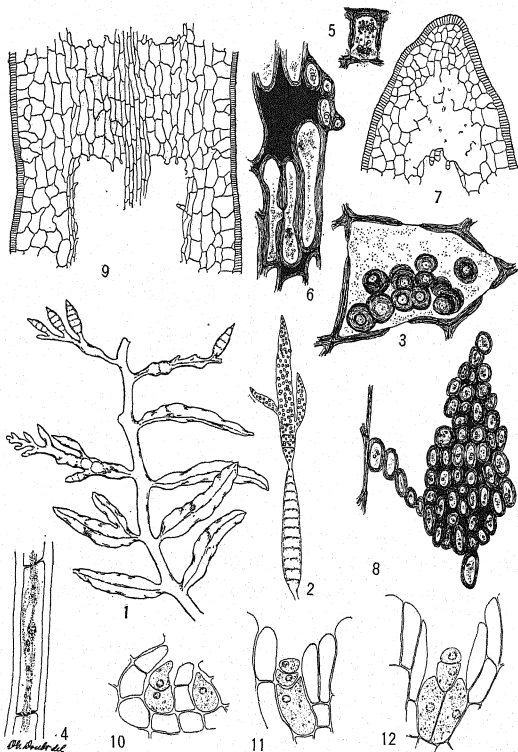
Both lighter and darker plastids exist in large numbers in the peripheral cells, where they sometimes fill the entire space; but generally they can be seen to be connected by protoplasmic strands with the nucleus in older cells, or lying free in the vacuolated protoplasm of cells nearer the apex, in which they are much fewer in number. They are fairly numerous in the wall cells of the conceptacles but very rare in the pith. Only highly refractive, nearly colorless plastids are found as a rule in the paraphyses cells, although near the ostiole some shorter ones are quite packed with brown plastids. Hyphae seldom have these darker plastids, nor do the mature cells of the midrib. In these latter cells particularly, and elsewhere to a less extent, are to be found granules, globules, and crystals of many kinds, which react variously to stains and frequently do not stain at all.

The material was examined for protoplasmic connections, and these were found in every place in which they were sought. The cells of the midrib had sieve plates (fig. 4), and the paraphyses, peripheral cells, hyphae, and isodiametric cells had pores through which protoplasmic strands were seen to pass. The pores in the peripheral cells were at the base in lateral walls, one in each wall; there is but one pore in a wall in every case where pores exist, so far as known. The pore in the basal wall of a peripheral cell is generally in the center, but its position varies (fig. 5).

The walls are composed of many striated layers of material, as a rule divided by an extremely narrow middle lamella. Very frequently the angles of the cell walls have loosened and pulled apart, in which case the intervening space is filled with mucilage (figs. 6, 7). The walls of the inner isodiametric cells are particularly thick, and through them the middle lamella seems to connect across the pore, as if it alone composed the actual pore wall. Walls of every cell except in the paraphyses and apical region are quite thick.

The nucleus was found to be uniformly oval in shape, and to average  $5\ \mu$  in diameter in every vegetative cell except those at the base of the apical groove. There they were larger, the apical nucleus





FIGS. 1-12.—Fig. 1, habit sketch showing intergrading between leaves and air vesicles, natural size; fig. 2, habit sketch of vesicular shoot terminated by receptacle, twice natural size; fig. 3, isodiametric cell containing fucoxanthin plastids; fig. 4, one of cells of midrib showing protoplasmic continuity through sieve plates; fig. 5, peripheral cell showing protoplasmic continuity through pores; fig. 6, lysigenous splitting of cells adjoining air chamber and entrance of hyphae elements; fig. 7, portion of cross-section of young air chamber showing breakdown of cell tissue; fig. 8, cross-section of midrib in chamber showing protruding filaments; fig. 9, longitudinal section of mature vesicular diaphragm showing condition of thallus cells and hyphae; fig. 10, single cell and 2-cell stages in development of conceptacle; fig. 11, 3-cell stage; fig. 12, 4-cell stage.<sup>1</sup>

<sup>1</sup> Drawings made with the Abbe camera lucida. Figs. 7, 9, 16, 17 magnified 150 times; figs. 6, 8, 650 times; figs. 3, 4, 5, 10, 11, 12, 13, 14, 15, 1250 times. The drawings have been reduced one-half.

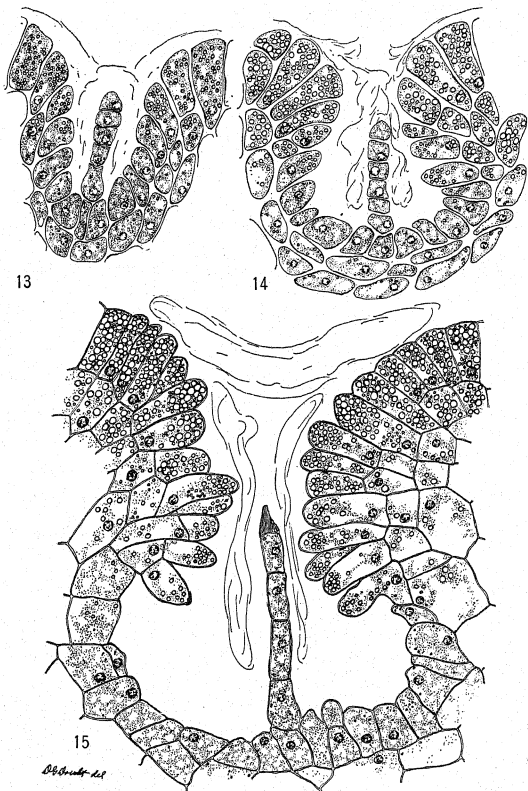
being nearly  $10\ \mu$  in diameter. There was only one nucleus in every living cell of the plant so far as examined. This includes the cells of the midrib. The nucleus tends to occupy the center of the cell, except in the peripheral layer, where it seems always to be pressed down toward the base by the quantities of plastid material contained in the cell. In peripheral cells near the apex the nucleus still occupies a central position in the cell.

Hyphae arise as papillae from marginal cells of air chambers, or from cells of the midrib. In either case they are usually rounded in shape until the second cross wall has formed, after which they become filamentous. Young paraphyses frequently develop in the same way.

Hyphae are not numerous in *H. dioica*. None exist in the receptacles, except in old ones where air chambers are present or in process of formation. In fact, no hyphae have been found in this species except in the formation of or adjacent to air chambers. Hyphae are not found in the newer portion of the plant, but arise only after the midrib is well developed and the isodiametric cells are losing vitality. In addition it may be said that air vesicles do not develop in the newer portion of the plant.

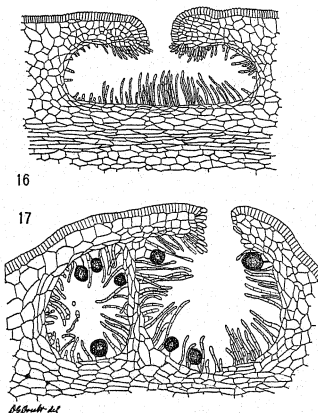
The conceptacle develops from a small initial cell, which becomes distinguishable about the third or fourth segment from the apical cell. Its base at that time is barely depressed below the neighboring cells, but the cell has taken on the characteristic tongue-shaped apex and is slightly shorter than the adjacent cells. Considerable mucilage is present in the apical groove, and separates the cells for several layers down into the thallus, so that no contact of adjacent cell walls is apparent near the base of the apical groove (fig. 10).

The conceptacle initial divides almost immediately, cutting off the tongue cell by a depressed U-shaped wall. Following that the basal cell merely elongates while the tongue cell divides twice horizontally (fig. 11). The adjacent cells are meanwhile growing and dividing, so that by the time the young conceptacle has been pressed upward to the top of the groove it is noticeably sunken in a small groove of its own, and the descendants of the tongue cell are free from the surrounding tissue. By this time a third wall has come in, separating the basal cell longitudinally into two equal parts, initiating the development of the wall of the conceptacle (fig. 12).



FIGS. 13-15.—Figs. 13, 14, further stages in development of hair and conceptacle wall; fig. 15, the hair and development of paraphyses in conceptacle.

Several successive divisions of basal and of tongue derivatives take place as the conceptacle recedes across the head of the shoot; and by the time it has reached the side of the receptacle the cells of tongue origin number five or six, and are depressed below the mouth of the young conceptacle by divisions of the basal cell. The cells at the base are becoming noticeably shorter and more numerous than



FIGS. 16, 17.—Fig. 16, later stage: paraphyses about half grown, and hair not distinguishable; fig. 17, oblique section of double, female conceptacle.

those near the mouth (figs. 13, 14). The basal cells have divided much more rapidly than their descendants and continue to broaden out the base.

As this trend of development continues, the mucilage which had been carried along in the conceptacle depression, between the walls of the adjacent cells, and particularly as a covering over the mouth of the depression, now becomes concentrated in small masses in the conceptacle and at its head, and it soon disappears. The tongue cell derivatives become elongated but give no evidence of further division, and the upper cell appears coated with mucilage. The rapidly

dividing basal cells begin to send out papillae and initiate paraphyses, while the more slowly growing cells of the neck, particularly the inner portion of them, loosen along their lateral walls and by successive divisions produce paraphyses also (fig. 15). After the paraphyses along the base of the conceptacle begin to grow there is no certain evidence of the tongue cell derivatives (fig. 16).

A peculiar condition has appeared frequently in this material. Two conceptacles develop fully, with their ostioles in different planes but with only three layers of cells separating them (fig. 17). Such a wall when seen in median section terminates abruptly at either end in a plane at right angles to the thallus. These double conceptacles are usually visible in habit material, as indicated in fig. 2.

*Ectocarpus acuminata* (17), previously described as a parasite growing in the conceptacles of *H. dioica*, was found quite frequently in the female conceptacles of this material. The sporangia were fully developed and beginning to shed their spores at the time of the extrusion of eggs from the conceptacle, and the vegetative cells were very few in number and scarcely distinguishable from cells of the rather abundant paraphyses. The spore nuclei were almost identical in size and appearance with those of the host plant.

### Discussion

In appearance the material used was essentially as described by GARDNER (6), except for its greater size; more widespread and numerous air chambers, and differentiation between male and female plants. Growth was apical and followed the description of OLTMANN (14) for *H. siliquosa*.

A study of the cell structure at once brought into evidence the plastid content, particularly the large dark plastids centering in the layer or two next under the peripheral layer. A comparison of the work of HANSTEEN, CZAPEK, and PALMER indicates that these plastids are what has been known as fucosan, and also that they contain fucoxanthin. Their size in excess of those in the peripheral layer may be accounted a result of food storage.

The existence of protoplasmic connections through the walls in various parts of the plant would agree with the findings of HICK in *Fucus* and *Ascophyllum*. Sieve plates had been found by WILLE in

some members of the Fucaceae, but LE TOUZE had disputed their existence in *Halidrys*. However, OLTMANNS and others considered that WILLE was correct. HANSTEEN also was not certain of protoplasmic connections through the pores of *Fucus*, *Pelvetia*, and *Sargassum*, but they were definitely distinguishable in the material of the present study of *H. dioica*.

The striated nature of the walls had not previously been noted. It was found to be a constant condition. It may be further remarked that the space left at the angles of the loosened cell walls had the appearance of mucilage rather than a much expanded middle lamella, as suggested by LE TOUZE. The idea proposed, that it is the middle lamella which connects across the pore opening, based on the results of staining, does not necessarily correlate with the statement of LE TOUZE that a portion of the original wall closes the pore, but depends on a determination of the origin of the middle lamella. The original wall may be involved in some way.

The lowered position of the nucleus toward the base in the peripheral cells is considerably more pronounced than the description of LE TOUZE for *H. siliquosa* would indicate. Also the existence of but one nucleus in the cells of the midrib is contrary to the report of OLTMANNS and WILLE for the Fucaceae in general, but coincides with the description of LE TOUZE for *H. siliquosa*. The larger size of the apical nucleus had not previously been noted. Otherwise the condition of the nucleus agreed with former descriptions.

The study of hyphae and air chambers showed, as has been found by previous workers, that they do not arise in the newer, more vigorous parts of the plant, but in regions which seem to be losing vitality. This loss of vitality may possibly be due to lack of food, or to pressure from some source or other, according to inferences made from this material. The fact that air vesicles do not arise in younger parts of the plant is attested, not only by the cell structure, but by the growth of deciduous receptacles, reported by GARDNER (6), above the air vesicles, and the fact that air vesicles never appear above receptacles. Moreover, the mucronate apices of air vesicles frequently contain normal apical cells, in which case early stages in the development of the conceptacle are to be found there, thus confirming the statement of AGARDH (1). But a majority of the vesicles

examined had been broken off, and either had the final chamber exposed or the organ had been grown over and the wound healed, as OLTMANNS described the recovery of wounded tissue in the *Fucaceae* (14).

Neither the exact cause of hyphae origin nor of the approximately periodic origin of air chambers has been investigated in previous studies. It is known that hyphae form a maze in the irregular mass of loosened disintegrated cell tissue. The cells of the midrib remaining in contact with the hyphae become looser, and while still rope-like, become divided into at least two strands. As observed in the present study, many horizontal hyphae filaments run out from these strands, connecting them with the receding hyphae, which now, like a wall, are proceeding where tissue breaks down but becoming attached where it holds (figs. 6-9). Since hyphae are conducting cells, according to WILLE, it may be supposed that they supply nourishment to the cells too distant from the outside or from light rays to acquire their own food, and thus hyphae maintain the cells in a stable condition, not subject to breakdown to any great extent.

It may be supposed with reason that though hyphae are conductors, the stimulus that causes them to start is chemical, and their first activity is resorption. If this be the case, another hyphae center will ultimately be formed a few cells above the now stable chamber, as a result of the breakdown of cell tissue at this spot. It is assumed that above a certain region the cells are not yet vulnerable to these new hyphae, and that on the other hand the basal diaphragm of the new chamber is already fairly well formed through the activities of the recent group of hyphae. The size of the new chamber then is predestined.

A study of the development of the conceptacle in this material brought out certain marked differences between some of its phases in this species and those which had previously been described for *H. siliquosa*. The initial cell agreed with the description of NIENBURG, in which he figures a tongue-shaped apex rather than the more or less rectangular cell of OLTMANNS (13) and BOWER. The first wall was curved, as NIENBURG has found, rather than according to the findings of BOWER or OLTMANNS, but there the resemblance to NIENBURG's schedule ceased. The second wall, as has been noted, came

in horizontally in the tongue cell, rather than vertically in the basal cell. Even if it could be assumed that a division took place in the basal cell in the plane of the section, and thus not visible at this juncture, it would have been contrary to the sequence of NIENBURG, as would the division of the upper cell. It also has no parallel in the schedules of BOWER or OLTMANNS. Not only is the second wall located differently, but also the third comes in in a different sequence. The third wall in *H. dioica* comes in vertically across the center of the basal cell. This was the second wall in NIENBURG's series and in OLTMANNS', while it had no correspondence to anything in BOWER's series. According to BOWER, by comparison, there might be two or more straight, horizontal divisions of the initial cell before the vertical divisions of the basal cell began; then the first two walls of the basal cell were inclined toward each other.

Certain similarities as well as contrasts were observed between the behavior of the young conceptacle in *H. dioica* and in *H. siliquosa*, as described by the three previous reports. All are agreed that at least the base of the conceptacle is lined by cells derived from the basal portion of the initial, and that these cells produce paraphyses and gametes. Also all are agreed that the upper portion of the initial divides four or five times horizontally, producing a hair. BOWER does claim that this hair is early thrown off by the swelling of the wall dividing it from the basal cell, whereas it is otherwise agreed that the fate of the hair is uncertain; that it may remain in the mature conceptacle, indistinguishable from the surrounding paraphyses. Also, BOWER and OLTMANNS are not certain but that the peripheral cells adjoining the initial cut off some if not a considerable number of the upper wall cells of the conceptacle. There seems to be no evidence of that in *H. dioica*.

One phase of the development of the paraphyses had not been touched upon before, the fact that the upper wall cells split laterally and grow out as paraphyses rather than forming papillae as an initial step. This splitting may be accounted for by the fact that the growth tendency is toward the ostiole, and does not press them as firmly as the cells are pressed on the base of the chamber (fig. 15).

Finally, the development of double conceptacles had not previously been noted. It seems likely that the situation in fig. 2 fre-



quently occurs, in which two successive initials are separated by only one wall. It seems reasonable to suppose that the pressure of growth exerted by these two young initials and their adjacent cells inhibited division in the vertical, though not in the horizontal nor longitudinal planes in the basal cell of this single wall layer; and that later, as the number of segments from the basal cell of the initial multiplied, they spread over the surface formed by this wall, which grew at a corresponding rate, and the outcome was a 3- or 4-layered diaphragm between the two conceptacles.

### Summary

1. There is an unbroken series of intergrades between a leaf and an air vesicle in *Halidrys dioica*.
2. The origin of air chambers seems to be a matter of food relationships in which hyphae play an important part.
3. The fucosan of HANSTEEN seems to be a fucoxanthin plastid.
4. Protoplasmic connections are continuous throughout the plant.
5. The early development of the conceptacle stresses the upper rather than the basal cell, as preceding work on the European species had shown.
6. The frequent appearance of double conceptacles may be due to the action of two initials upon a single intervening wall cell.

I am indebted to PROFESSOR C. J. CHAMBERLAIN for the material used in this study, and also for suggestions in regard to the work.

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## EFFECT OF SULPHATE ON LEMON LEAVES<sup>1</sup>

A. R. C. HAAS AND E. E. THOMAS

(WITH TWO FIGURES)

It is well known that the irrigation waters of citrus areas contain more or less salts; even those used on the very best citrus groves contain ordinarily some chloride and sulphate (6). These in small to moderate amounts are not particularly objectionable, but large amounts of chloride or sulphate not only bring about the accumulation of toxic concentrations within the leaves, but also reduce the absorption of nitrate by the plant (3).

Analyses of lemon leaves from soils irrigated with water containing large amounts of calcium sulphate have shown that severe injury by accumulation of sulphur compounds in the leaves is accompanied by a reduced amount of total phosphorus, represented almost entirely by the decrease in inorganic phosphorus. Conversely, the toxicity can be somewhat decreased by generous additions of nitrogen fertilizers. These bring about a greater leaf area with a consequent reduction of the concentration and toxicity of chloride or sulphate in the leaves.

The occurrence of large amounts of chloride or sulphate in the irrigation water is accompanied by high concentrations of sodium, calcium, or magnesium. The effects of the accompanying bases are the more favorable when the bases are divalent rather than monovalent. Often the choice of fertilizer materials is such that considerable amounts of chloride or sulphate are applied either unintentionally or without knowledge of the danger of injury that may ultimately result.

Experiments have been conducted with the use of sand cultures in order to observe the effects of sulphate upon the growth of citrus. Twelve ten-gallon crocks with a hole in the bottom for drainage were employed as the culture vessels. Crushed quartz was placed in the bottom of each crock, and pure silica sand containing one

<sup>1</sup> Paper no. 186, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

pound of pure calcium sulphate was placed in each crock. Budded lemon trees washed free of adhering soil were planted in the sand after being pruned free of all foliage and most of the smaller roots. The trees were allowed to grow in these containers while receiving a culture solution identical with that given to series I in table I. Iron in the form of ferric tartrate, and a solution of "A-Z" (2) were added to the culture solutions, so as to give a concentration of 0.2 ppm of certain constituents.

Instead of producing normal growth as in controls that received Hoagland's culture solution, many of the trees lost a considerable

TABLE I  
SAND CULTURES: CULTURE SOLUTION ADDED TO SAND CONTAINING  
CALCIUM SULPHATE

SERIES	PARTS PER MILLION IN CULTURE SOLUTION ADDED						
	Na	K	Ca	Mg	NO <sub>3</sub>	SO <sub>4</sub>	PO <sub>4</sub>
I.....	61	22	.....	57	164	223	35
II.....	61	22	477	57	1643	223	35
III.....	61	22	795	57	2629	223	35

number of their leaves at the end of the first year of growth, so that the shoots were bare of leaves except near the tips. In some cases the entire shoots were defoliated, and some of the leaves turned greenish yellow, while others mottled somewhat and became yellow or golden colored at the tip prior to abscission. A composite sample of mature leaves from all twelve of these trees was taken for analysis.

The twelve containers were then arranged in three series, which received the culture solutions described in table I. The calcium sulphate served as the only source of calcium in the first series of four crocks, while in the other two series of four crocks each the culture solutions contained additional calcium as calcium nitrate. The pH of the solutions added was close to 7, and that of the drainage water ranged from pH 4.8 to pH 6.6. Acidity of the culture solution as a result of absorption or excretion by the roots and the possibility of its toxic action upon growth are therefore factors involved in studies pertaining to calcium sulphate. We have eliminated much

of this acid toxicity by the frequent use of solutions of high pH values.

In series II and III the leaves were retained on the shoots for much longer periods than in series I, although gradually much of the distal portion of the shoots became bare of leaves. In series II and III leaves of a certain type were affected by mottling with a yellowish or golden colored leaf tip (fig. 1). As the leaves became mature, the yellowish mottling tended to become somewhat bronzed. Also brown spots dotted the margin and the mottled area on the ventral side of the leaves. In some cases actual burning or drying out of the margin of the leaves occurred. These effects of sulphate resemble those caused by an excess of boron. The latter, briefly alluded to in a preliminary announcement by KELLEY and BROWN (4), will be more fully discussed by them in the near future.

Analysis of a composite sample of mature leaves from the trees, before dividing the containers into three series, showed that the dry matter of the leaves contained 1.57 per cent of sulphur, which is equivalent to 4.70 per cent of  $\text{SO}_4$ . The ash of the water-soluble fraction was 8.92 per cent, and that of the water-insoluble fraction was 10.10 per cent of the dry matter. The ash of the water-soluble fraction was 46.89 per cent of the total ash of the leaves, which is quite abnormally high for mature lemon leaves. HAAS (1) has shown the ash of the water-soluble fraction of normal, mature lemon leaves from the plots on the Rubidoux tract of the Citrus Experiment Station to be about 30 per cent of the total ash.

The total calcium of the lemon leaves of the trees in the original calcium sulphate series was 27.22 per cent of the total ash, which is typical of mottled leaves (5). The calcium of the water-soluble fraction of normal lemon leaves has been found by HAAS (1) to be about 17-18 per cent of the total calcium, whereas 30.93 per cent of the total calcium of these lemon leaves was water soluble. The magnesium, sodium, and potassium percentages were about the same as for normal lemon leaves. It is quite possible that the high content of sulphur in the leaves may have brought about an increased solubility of the calcium because of increased acidity of the tissue, although such sap studies have not been made as yet.

In the sand cultures just described it was observed that, with

increasing concentrations of calcium nitrate in the culture solution, the lemon leaves presented a darker green color than where no addi-

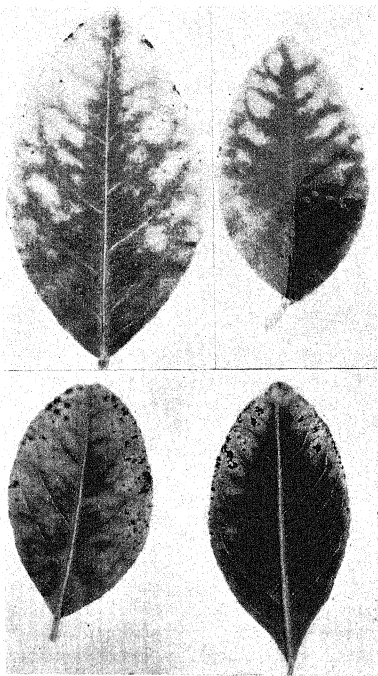


FIG. 1.—Effects produced upon lemon leaves from trees in sand cultures containing large concentrations of sulphur: upper row, dorsal surface; lower row, ventral surface.

tions of calcium nitrate were made. The trees to which calcium nitrate was added made fair growth and retained their leaves, while

those that did not receive the calcium nitrate made practically no growth and lost nearly all of their leaves. There is reason to believe that the absorption in increasing amounts of any other anion will reduce the toxic effect of the absorbed  $\text{SO}_4$ . This was quite evident from the effect on lemon trees in several series of sand cultures in galvanized iron containers 20 inches in diameter and 26 inches deep. In one case 500 gm. of calcium sulphate was mixed with the sand in each container, and in another case 500 gm. of calcium sulphate plus 500 gm. tricalcium phosphate (table II).

TABLE II

SAND CULTURES WITH NUTRIENT SOLUTION AND ADDED SALTS

ADDITIONS TO SAND	PARTS PER MILLION IN CULTURE SOLUTION ADDED TO SAND							
	Na	K	Ca	Mg	Cl	$\text{NO}_3$	$\text{SO}_4$	$\text{PO}_4$
$\text{CaSO}_4$ .....	7	184	.....	54	10	225	216	105
$\text{CaSO}_4 + \text{Ca}_3(\text{PO}_4)_2$ .....	7	142	.....	54	10	225	216	.....

Each series of sand cultures included budded lemon trees, and received all of the elements necessary for growth. In the calcium sulphate series the leaves were a bronzed greenish yellow, with many of the shoots bare of leaves. Where tricalcium phosphate and calcium sulphate were added to the sand the leaves were somewhat mottled, as were those in the preceding experiment (fig. 1). The trees grown in the first series made practically no growth and lost nearly all of their leaves, while those in the second series made very good growth and retained their leaves.

It was observed that in many of the fertilizer plots of the Rubidoux tract of the Citrus Experiment Station, the lemon shoots had lost many of their oldest leaves; and many of the leaves still attached were almost indistinguishable from the leaves of trees which received a large amount of sulphur in sand cultures. Moreover lemon trees on unfertilized plots showed no such effects. At once it was surmised that possibly accumulations of sulphur had occurred in the leaves.

The concentration of  $\text{SO}_4$  in the soil solution in the various plots is subject to considerable change throughout the year by rainfall,

irrigation, and cultural operations, as well as by root absorption from the soil solution. It is of interest that the lemon leaves from trees of plots D, E, J, K, N, and R, which received applications of fertilizers containing sulphate,<sup>2</sup> showed visible evidence of injurious effects, while lemon leaves from trees on the unfertilized plots B and M showed no obvious symptoms. It is of added interest that lemon leaves from trees on plots A, L, and Q, which received as much if not more  $\text{SO}_4$  than some of the other plots, showed no symptoms of sulphate accumulation.

Analyses were made of mature lemon leaves collected in August, 1927, from the various fertilizer plots of the Rubidoux tract. Total chlorine was determined by the sodium carbonate method, and total sulphur by the magnesium nitrate method. The results are given in table III, each percentage representing the average of two or more closely agreeing duplicate determinations.

It will be noted that the chlorine concentration is nowhere excessive, and that the dry matter of lemon leaves from trees of plots A, L, and Q contains approximately the same amount of sulphur as that from plots B and M. The concentrations of sulphur found in lemon leaves from trees in plots A, L, Q, B, and M agree very well with the results obtained by KELLEY and CUMMINS (5) for normal mature lemon leaves. The dry matter of the lemon leaves from trees of plots D, E, J, K, N, and R, however, contains more sulphur in each case. Fig. 2 shows lemon leaves in which the accumulation of sulphur was noted.

In examining table III the question may be raised as to why the lemon leaves of trees on plots A, L, and Q show such a low concentration of sulphur and consequent lack of injury, in view of the fact that considerable sulphur is added in their fertilizer treatment. This but emphasizes an important consideration mentioned at the outset, namely, that additions of fertilizers that may introduce large amounts of  $\text{SO}_4$  ions in the soil solution may bring about considerable injury (plots D, E, J, K, N, and R). If such fertilizers be supplemented by fertilizers that add large amounts of ions other than sulphur to the soil solution, these latter tend to annul some or all

<sup>2</sup> A water extract of "fine steamed bone meal" used on plot E showed copious amounts of sulphate.



of the bad effects that otherwise would have been produced. The addition, therefore, of certain fertilizers that supply nitrate, etc., to soils rich in sulphur may have their beneficial effect partly by causing the trees to produce more growth, with a consequent reduction of the sulphur in the leaves to the point where it has no toxic effect. Growth of new leaves, therefore, acts to dilute and reduce the toxicity of sulphur within the leaves.

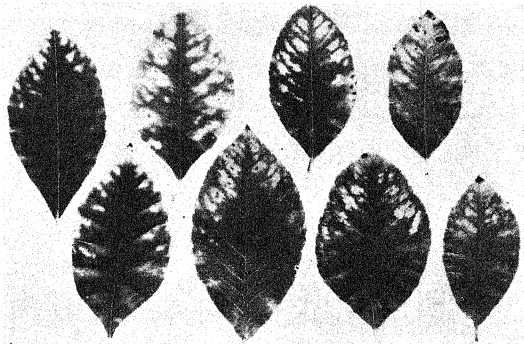


FIG. 2.—Discoloration of lemon leaves that accompanies sulphur accumulation; leaves taken from Rubidoux Tract, Citrus Experiment Station.

This discussion may bring a partial answer to the oft repeated question as to what concentration of nitrate shall be maintained in the soil solution for the best condition of citrus. There seems to be no one definite concentration of nitrate in a soil solution that can be said to be best for citrus on any soil type, or on the same soil type under different types of fertilization. The concentration of nitrate or any other constituent that is most desirable in the soil solution is dependent, in part at least, on the concentration of the other constituents of the soil solution and upon their ease of displacement and rate of renewal.

In certain lemon-producing districts it has long been known that



the irrigation water is comparatively rich in sulphur, largely in the form of calcium sulphate. Frequently leaves of such lemon trees are tip-burned, and have a type of mottling resembling that in fig. 1. Leaves from one location showed the total sulphur calculated as  $\text{SO}_4$  to be 3.14 per cent of the dry matter, while ordinarily the normal leaves of good groves contain about 0.96 per cent. The inorganic  $\text{SO}_4$  constituted 12.89 per cent of the ash, or 2.52 per cent of the dry matter; while the Ca was 32.6 per cent of the ash, or 6.38 per cent of the dry matter. Leaves from another location contained 9.09 per cent of  $\text{SO}_4$  and 23.78 per cent of Ca in the ash. The total sulphur calculated as  $\text{SO}_4$  was 1.80 per cent of the dry matter, while the Ca was 3.30 per cent. The leaves were of full size but severely mottled and tip-burned, the mottling being accompanied by a bronzed coloration. These results indicate that accumulations of sulphur may occur in lemon leaves when the trees are irrigated with water containing appreciable amounts of sulphate.

### Summary

1. In controlled sand cultures it was found that large concentrations of sulphate in the solution bathing lemon tree roots may bring about toxic effects in the leaves.
2. These effects are characterized by a type of mottling with a yellow or bronzed coloration and sometimes burning of these areas. This is accompanied by marked abscission of the leaves.
3. In sand cultures a reduction in toxicity due to sulphate was brought about by increased concentrations of phosphate and nitrate.
4. Field investigations upon lemon leaves have confirmed observations previously made with sand cultures, and have shown the ameliorating effect of supplementary fertilization that brings about increased tree growth with a reduction of the concentration of accumulations within the leaves to a point where toxicity is not evident.
5. Presumably there is no one general optimum concentration of nitrate for the growth of citrus. The desirable concentration depends upon many factors, such as the concentration of other constituents of the soil solution and their ease of renewal.

6. Irrigation supplies that are rich in sulphate may cause more or less injury to lemon trees, especially where the available nitrogen is insufficient.

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## INJECTION METHOD AS A MEANS OF IMPROVING CHLOROTIC ORANGE TREES<sup>1</sup>

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(WITH THREE FIGURES)

Chlorosis of citrus trees, although widespread throughout the citrus-growing areas of California, is not of a very serious nature except in restricted localities. Its occurrence may be associated with various soil conditions, but frequently the complications are such that it is difficult to determine the causal factor. The chlorosis of citrus often occurs on soils that are rich in calcium carbonate or other carbonates. Such soils usually have a high enough hydroxyl-ion concentration to precipitate out in the soil or within the plant the iron salts so essential for the production of chlorophyll. Frequently such soils are rich in the constituents necessary for growth, but some of them are made unavailable for the normal growth of the plant. In such areas the drainage conditions may be of the very best, and yet chlorotic growth appears as a consequence of the alkalinity due to the calcium carbonate.

Our first effort to remedy chlorosis in citrus plantings was confined to lemon trees, where large applications of iron sulphate were applied directly to the soil. The applications varied in amounts from 6 to 25 pounds per tree, and the material was applied in various ways, namely, in holes, in furrows, and in basins where the surface soil was temporarily removed. An adequate number of chlorotic lemon trees were selected as controls, and kept under observation during the time of the experiment. Observations were made at intervals over a period of five years, and the conclusion was reached that no definite improvement of the trees could be attributed to the applications that were made.

Further observations have been made on orange trees growing on soil which is rich in calcium carbonate. Here the applications of iron sulphate have been made in amounts varying from 12½ to

<sup>1</sup> Paper no. 184, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

100 pounds per tree. The material was broadcast and thoroughly incorporated with the surface soil. No obvious improvement of the trees as a consequence of the treatment could be noted.

The writers have recently limited their experiments on chlorosis almost entirely to the injection method as a means of treating Valencia orange trees on soils known to have a high calcium carbonate content.

The method employed is in many respects similar to that previously employed by GORDON and LIPMAN (1) and LIPMAN and GORDON (3, 4), but with certain modifications. In their method a single hole was bored into the trunk of the tree, almost at right angles to the direction of the trunk, and a glass tube was sealed into the hole with wax. This glass tube was attached by means of a long rubber tube to the reservoir containing the solution.

In our method we have substituted a galvanized iron pipe for the glass tube. This iron pipe is threaded at one end, screwed into the hole for a short distance and sealed by means of beeswax.

In our initial experiments with this method we employed but one hole for the injection of the solution into the tree, and iron tartrate as well as iron sulphate was used. Whenever chlorotic trees were injected, adjacent chlorotic trees were kept under observation as controls. This is a very important consideration when trees are observed over a period of years, in order that any improvement that may result from causes other than those brought about by the injection may be evident.

Injection experiments have been made on Valencia orange trees at different seasons of the year, and it has been found that the greatest response to the treatment occurs when it is given just prior to the beginning of new growth in the spring.

Previous investigators (MILAD 6, and MARSH and SHIVE 5) have found no deficiency of iron in chlorotic plants, but rather a non-uniform distribution of that which is present. In order to determine whether the iron present in chlorotic citrus trees can be made soluble and be utilized by the chlorotic foliage, therefore, we have injected into branches of chlorotic orange trees solutions of citric and of tartaric acid, in concentrations ranging from 1 to 9 gm. in two liters of distilled water, with no positive results.

Valencia orange trees about 20 years of age were each injected by the single-hole method with two liters of distilled water containing 10 gm. of iron tartrate. As a result of this single injection there was a marked abscission of the older chlorotic leaves, followed by a production of new growth from the axillary buds. The new growth was of normal green color and a considerable increase in the fruit production was observed. This immediate improvement in the condition of the trees confirms the results of LIPMAN and GORDON (3). Frequently, however, a single branch in a tree was conspicuous in that it showed no effect of the treatment. In less than two years the leaves of the succeeding cycles of growth were of a pale color, indicating the transient nature of the effect.

By the single-hole method we have also injected Valencia orange trees in the same grove with 3, 5, or 7 gm. of iron sulphate in two liters of distilled water. In these experiments, in contrast to those in which iron tartrate was used, the foliage of those branches only that were above the hole showed the effects of the treatment.

In the case of iron sulphate, there was not as marked an abscission of the older chlorotic leaves as in the case of iron tartrate, and the apparent improvement of the tree appeared to be more gradual, since the old chlorotic leaves were retained for a much longer time.

As a result of these observations it was evident that, in order to affect the entire tree by these iron salts, it would be necessary to inject the solution either in or below each main branch. Experiments were therefore begun in which a hole was bored below each main branch. Two liters of solution containing 1-2 gm. of iron tartrate was injected below each main branch. The trees employed had from three to five main branches. Subsequent observations showed that such treatment was very effective. There was marked abscission of the older chlorotic leaves, and the new growth produced had the green color of normal leaves. The subsequent cycles of growth, however, became increasingly chlorotic.

A similar experiment with iron sulphate was conducted in the same grove. Two liters of solution containing 1.5-2 gm. of iron sulphate was injected below each main branch. The trees selected had from three to seven main branches. As already stated, the effect on the trees was much less marked than with similar concentrations

of iron tartrate, but the trees showed considerable improvement in color as the old chlorotic leaves gradually abscised. The improvement of the trees was of comparatively short duration, in that succeeding cycles of growth became increasingly chlorotic.

Since the effect of the treatment was found to be temporary it became necessary to reinject the trees. It was found impossible to reinject them with solution by making use of the holes previously employed. This was true whether free circulation of air was permitted into the holes or whether the holes were sealed after the first injection and then reopened for the second treatment. The failure of the tree to absorb solution from the second application in the same hole bore no relationship to the concentration of the solution employed. It has been impossible to obtain absorption in the same hole from successive injections of water or of weak concentrations of iron solutions. However, if the hole is enlarged, some further absorption of solution takes place, but this may be extremely small in amount and insufficient to prevent the foliage from again becoming chlorotic. If new holes are bored on the same horizontal plane as that of the original holes, more solution can be absorbed by the tree with beneficial results. After making several applications in this way, it becomes necessary to bore new holes in the same longitudinal plane as that of some previously bored. In this case we have found that it is difficult if not almost impossible to secure absorption from these new holes. This is true whether the holes are placed above or below the original one.

An investigation as to the cause of the lack of absorption from new holes made in the same longitudinal plane as those previously used was then conducted. On boring the new holes it was noticed that the wood was of a darker color than that of untreated trees. Trees injected in this way were sawed off at the ground level and the trunk and lower portions of the main branches were then cut in various planes. Fig. 1 illustrates two transverse sections of the trunk of an injected tree. It shows the callusing-over of a hole shallower than that ordinarily employed. Even though the exterior of the hole was immediately sealed after the injection was completed, the inside of the hole is almost completely filled with fungus. The hole is bounded by a narrow margin of dead and discolored tissue.



The figure shows the location of four shallow holes, and shows that there is very little lateral movement of the injected solution; this condition explains the fact that only those branches above the hole are affected by the injection.

A longitudinal section of the trunk of a Valencia orange tree that has been twice injected in new holes in the same vertical plane is illustrated in fig. 2. The discoloration of the tissue brought about by the injection of the iron-salt solutions is very conspicuous. The

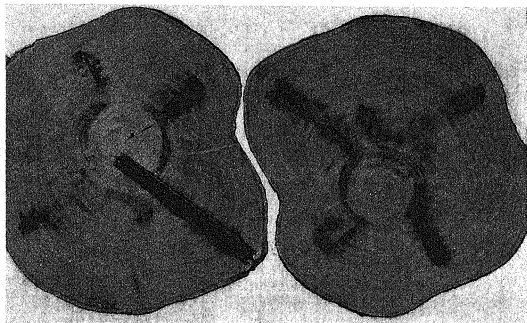


FIG. 1.—Transverse sections of trunk of injected orange tree, showing callusing over of hole and discoloration of wood in longitudinal plane brought about by injection of iron-salt solutions.

plane parallel to the long axis of the holes shows the marked upward and downward movement of the solution. This same plane shown in the photograph is perpendicular to other holes, and shows the small lateral movement of the injected solution.

The upward and downward movement of the injected iron solutions (fig. 2) is so pronounced that its effects frequently are visible in the main branches of the tree, as is well illustrated in fig. 3. A line connecting the darker areas in this transverse section lies in the same longitudinal plane as the hole in the trunk of the tree.

When concentrations of iron salts such as we have employed are used, no symptoms of internal injury are manifest on the ex-

terior of the trunk. With higher concentrations, however, a strip of bark may be killed for considerable distances up the tree. This killing of the bark is accompanied by an exudation of gum.

We have found in these studies that a concentration of 1.5 gm. of iron sulphate in two liters of distilled water has proved beneficial to chlorotic trees, while much smaller concentrations of iron sulphate have not proved effective in severe cases of chlorosis. This concentration, however, has produced the injurious effects described.

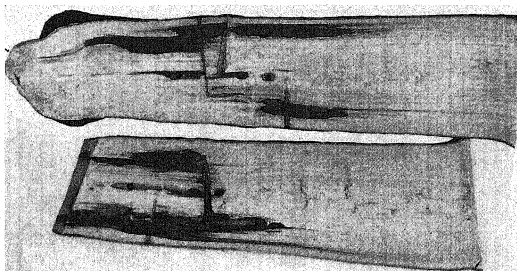


FIG. 2.—Longitudinal sections of orange tree trunk injected with iron-salt solution; discoloration shows the upward, downward, and slight lateral movement of iron-salt solutions.

It is of interest, therefore, to ascertain what effect an injection of 1.5 gm. of iron sulphate would have upon the tree when the salt was dissolved in 36 rather than 2 liters of distilled water. This experiment has shown that an orange tree 20 years of age could absorb but 18 liters of such a solution. Efforts to secure further absorption in the same hole, or in holes above or below the original hole but in the same vertical plane, have proved futile. An examination of the wood has shown a discoloration characteristic of iron salts.

This discoloration of the wood is in decided contrast with the normal color which persists after an injection of salt solutions other than those of iron. This was found to be the case in studies on the effect produced by the injection of large volumes (18 liters or more) of double-strength Hoagland's solution, and also by the injection of large volumes (6 liters or more) of calcium nitrate solution (800

parts per million of calcium) into badly mottled orange trees. No effects of such treatments were apparent.

The results we have obtained by the use of the injection method in the treatment of chlorotic orange trees confirm those reported by LIPMAN and GORDON (3) for lemon trees, so far as the initial effect upon the trees is concerned. Their conclusions, however, in regard to the injection method as a cure for chlorosis of lemon trees have not been confirmed by our results with orange trees.

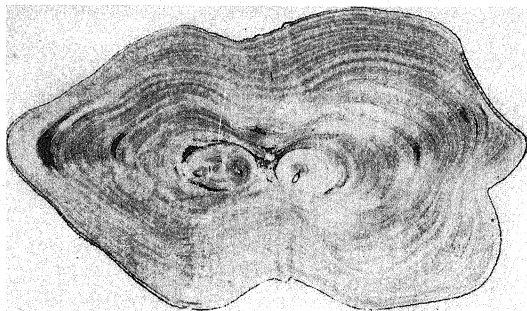


FIG. 3.—Transverse section of main branches of injected orange tree immediately above the trunk, showing pronounced upward movement of injected iron-salt solution.

In 1921 HENDRICKSON (2) injected pear trees growing on calcareous soil with iron sulphate solution through an inserted tube. After using various concentrations, he decided that without further work tree injection could not be recommended as a practical control for chlorosis.

In view of the temporary nature of the benefits to be derived from injection of orange trees with iron-salt solutions, the killing of certain tissues of the trunk with the possible accompaniment of fungous infection, the weakening of the tree, and the expense of such treatment, further investigation is necessary before tree injections with iron salts may be recommended as a cure for chlorosis of orange trees.

### Summary

1. Chlorotic orange trees have been improved temporarily by the injection of iron-salt solutions into the trees. Subsequent injections are necessary in order to maintain the trees in a healthy condition. The difficulty, however, lies in the fact that the same hole cannot be used for the subsequent injections. This is due to the fact that iron salts in the concentration required to improve the tree discolor and kill the wood for a considerable distance up and down the trunk.

2. In addition to the weakening of the tree, it is very likely that fungi will develop in the injection holes.

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## CURRENT LITERATURE

### BOOK REVIEWS

#### Gasteromycetes of eastern North America

A comprehensive, modernized treatment of American Gasteromycetes has long been needed. MORGAN's work is incomplete, it is not readily available to many workers, and his nomenclature is obsolete. The same criticisms apply to the writings of LLOYD and of PECK. The beautifully illustrated and very complete account of the Gasteromycetes of the eastern United States and Canada by COKER and COUCH,<sup>1</sup> therefore, will be eagerly welcomed by students of the fungi everywhere, but especially by those residing in the region covered or working on its fungus flora. While this region is not clearly delimited, it apparently includes continental North America east of the great plains. Over 150 species are recognized as occurring within this territory, and are described fully. There are, in addition, numerous critical notes on extra-limital species which add greatly to the value of the work.

The taxonomic treatment is conservative. One new genus, *Nigropogon*, belonging to the Hymenogastraceae, and 11 new species and varieties, mostly included in the same little known family, are described. This is surely not excessive in a work of the scope of the one under consideration. The Arachniaceae is apparently here proposed as a family for the first time, although the fact is not indicated. The authors suggest that *Astraeus* might well justify another family; but in this case, as in the instance of several doubtful species where new names could easily have been defended, they exercise commendable restraint. Had other authors been equally considerate, the problem of dealing with synonyms would be simpler.

The plates include reproductions of photographs, photomicrographs, and drawings, and illustrate both habit and microscopical details of the great majority of the species discussed. A few of the photomicrographs add little to the value of the book, but with this qualification, the illustrations represent a notable contribution to the morphology as well as to the taxonomy of the group.

The authors nowhere suggest the taxonomic rank they would assign to the Gasteromycetes as a whole; they recognize no subdivision larger than a family, and the arrangement of the families sheds little light upon their views concerning inter-relationships within the group. While comments bearing upon this problem occur here and there throughout the course of the systematic treat-

<sup>1</sup> COKER, WM. C., and COUCH, J. N., The Gasteromycetes of the eastern United States and Canada. 4vo. pp. ix+195. pls. 123. Chapel Hill, University of North Carolina Press. 1928.

ment, it is to be regretted that they have not seen fit to summarize their conclusions. The reviewer is inclined to characterize this lack as the one really disappointing feature of the book. A few details are subject to criticism. For example, the habitual reference to the basidiocarp as the "plant" has a certain amount of usage to justify it, but little else. The statement that MORGAN's specimens have been destroyed is fortunately incorrect. These have nearly all been preserved, and are now in the possession of the State University of Iowa, where, it is expected, they will soon be available for reference. The statement concerning the liberation of the spores of the Nidulariaceae (p. 131) is pure assumption. There is no evidence that in the natural course of events the spores of these fungi are ever liberated from the peridioles. In a few cases the keys do not seem to be clear. These are minor points, however, and it would be unfair to stress them in discussing a work which includes such a wealth of new and critical information concerning nearly every species discussed as to make it one of the outstanding contributions to mycological literature of recent years.

The bibliography seems to be complete, and, as in the case of the earlier works on the water molds and the Clavarias from the same source, the volume is beautifully printed, and remarkably free from typographical errors.—G. W. MARTIN.

#### NOTES FOR STUDENTS

**Metaxenia.**—An interesting and clearly written article by NIXON<sup>2</sup> tells of his studies upon the immediate influence of pollen upon fruit size and time of ripening in the date palm. Experiments were carried on at Indio, California, using trees of the Deglet Noor variety as seed parents, and employing pollen from other varieties of *Phoenix dactylifera* and also pollen from *P. canariensis*. An ingenious technique was developed which permitted the use of various pollens on different strands of the same ovulate inflorescence. Fruits which had been pollinated with "Fard no. 4" colored about two weeks earlier than those with the pollen parent "Mosque." A striking display was produced where the two were side by side on the same bunch. By varying the pollen employed the experimenter was able to modify the size of fruit and time of ripening, but not the flavor or sugar content. In explanation of this immediate influence of pollen NIXON refers to papers by SWINGLE, read at the International Congress of Plant Sciences in 1926, and to a paper<sup>3</sup> by the same author just published. SWINGLE suggests that a hormone-like substance may be secreted by the development of endosperm or embryo, which, on reaching the ovary wall, influences further growth and development. SWINGLE thinks that metaxenia is not limited to the date palm but probably occurs in many plants, and that it may be exerted upon tissues outside of the seed and fruit. As knowledge of metaxenia develops it may be of great value to the horticulturist.—F. RAMALEY.

<sup>2</sup> NIXON, R. W., Immediate influence of pollen. Jour. Heredity 19:241-255. 1928.

<sup>3</sup> SWINGLE, W. T., Metaxenia in the date palm. Jour. Heredity 19:257-268. 1928.

# THE BOTANICAL GAZETTE

December 1928

## NUCLEAR FORM AS RELATED TO FUNCTIONAL ACTIVITIES OF NORMAL AND PATHOLOGICAL CELLS

BESSIE GOLDSTEIN

(WITH ELEVEN FIGURES)

### Introduction

There is considerable evidence that the relations of the nucleus to metabolic activities involve its assumption of irregular forms, which have been generally interpreted as having their significance in the resulting increase of nuclear surface in relation to the cytoplasm. The nuclei of cells infected by fungi frequently are found to be hypertrophied, amoeboid, or lobed in outline, and this is regarded as direct evidence of pathological changes in the cell metabolism. Perhaps the most outstanding and fully studied case of abnormal nuclear form, either in plant or animal literature, is that of the nuclei of the giant cancer cells, which are extraordinarily hypertrophied, exceedingly irregular and deeply lobed, and have been said to multiply amitotically, by budding or by fragmentation.

While making a cytological study of certain variegated plants, in which this condition is assumed not to be induced by viruses, the writer fixed and sectioned for study material from *Lilium longiflorum* and its variegated form *L. longiflorum foliis albomarginatis*. Lily tissues are well known as favorable material for cytological study, and in certain cells in various parts of the plants very irregular nuclear forms were found.

Other material from both healthy and diseased lily plants was fixed and sectioned for study, and a wide range of nuclear types was found. These types included giant hypertrophied rounded nuclei; giant and minute so-called amoeboid nuclei; giant lobed or lobulate nuclei; nuclei with surfaces projecting outward as pointed teeth, horns, and rounded knobs; nuclei with so-called canals and furrows; nuclei apparently dividing by budding and constriction, and in all stages of apparent fragmentation, chromatin degeneration, and shrinkage. The tissues of some 70 different specimens of lily plants, including 20 varieties, have been studied. The material was obtained at the New York Botanical Gardens, R. A. HARPER's farm at Ridgewood, New Jersey, the florist shops of the city, and the greenhouses at Columbia University. In a preliminary way plants have been classed as healthy if they were of normal size, with a strong green leaf coloring, if they bore flowers and well developed fruits, and showed no perceptible yellowing or streaking of the leaves. Leaves turning yellow at the base of the plant were not necessarily considered as indicating a pathological condition. Plants suspected of being affected with mosaic because of the presence of light green or yellow green areas or streaks in the leaves were classed as pathological. Other pathological material was taken from plants which were stunted, dwarfed, or showed yellowing, whether due to poor cultural conditions or a specific pathogen.

For a study of the nucleus and its relation to metabolic activity in storage organs, apparently healthy material was used from the tubers of dahlia; the bulbs of hyacinth and narcissus; the cotyledons of sprouting soy beans, bush beans, and peas; and the endosperm and embryo of buckwheat, oat, corn, and castor bean. The material was fixed with either Flemming's medium strong solution, or with Allen's B 15 solution. The latter fixative gave most excellent results, especially for the nuclei. Sections were cut at  $5\mu$  or  $7.5\mu$  and stained with Flemming's triple stains.

Tables I and II summarize data from the literature as to the apparent relation of tissue differentiation to nuclear form. I have included nuclei said to be dividing amitotically with other cases of lobed or constricted types.



TABLE I

OUTLINE OF DATA RELATING METABOLIC ACTIVITY TO NUCLEAR FORM

AUTHOR	DATE	PLANT OR ANIMAL STUDIED	TISSUE OR ORGAN CONCERNED	NUCLEAR FORM
Korschelt.....	1884	Chironomus	Salivary glands	Irregular, lobed, amoeboid, toothed?
Howell.....	1889	Insects	Egg nurse cells	Amoeboid
.....	1890	Young kitten	Developing bone	Lobed, fragmenting
Müller.....	1892	Man	Cancer cells	Irregular, fragmenting, degenerating
Korschelt.....	1896	Insects	Spinning glands	Branched network
.....	.....	Crustacea	Salivary glands	Branched network
Foot.....	1897	Animal	Yolk nucleus	Amoeboid
Schnewind-Thies....	1897	Monocotyledonous plants	Nectaries	Lobed, incised, branched
Molisch.....	1899	Musa, chinensis	Latex ducts	"Blasenkerne"
.....	.....	Lycoris radiata	Slime ducts	"Fadenkerne"
.....	.....	Aloe	Secretory cells	Giant, lobulate
Torrey.....	1902	Zea mays, other grasses	Epidermal layer of the scutellum	Markedly lobed
Bashford and Murray	1904	Cat, mouse	Carcinoma, epithelioma	Amoeboid, amitotic
Reed.....	1904	Zea mays	Epidermal cells of scutellum	Distorted, swollen
Unna.....	1905	Man	Carcinoma	Lobed, fragmenting
Schmid.....	1906	Scrophulariaceae	Micropyle haustoria	Hypertrophied, fragmenting, amoeboid
Huss.....	1906	Ranunculaceae, Papaveraceae	Antipodal cells of embryo sac	Hypertrophied, fragmenting, amoeboid
Guilliermond.....	1908	Wheat, barley, corn	Cells of embryo	Lobed
Friedsohn.....	1910	Frog	Polymorphic, leucocytes, etc.	Lobed, fragmenting
Němec.....	1910	Corydalis cava, Secale cereale, Colutea arborescens	Endosperm	Abnormally large, amoeboid, fragmenting
Wright.....	1910	Euphorbiaceae	Latex tubes	Huge, lobulate
.....	.....	Cat, dog, mouse, rabbit, guinea pig, man	Megakaryocytes of bone marrow	Irregular, lobed
Szily.....	1911	Animal	Pigmented eye coat, Choroid sarcoma	Polymorphic
Maximov.....	1913	Dog, rat, man, guinea pig	Mastleucocytes	Polymorphic
Samuelson.....	1913	Empetrum nigrum	Endosperm haustoria	Hypertrophied, amitotic, minute
Schürhoff.....	1915	Ranunculus acris	Giant endosperm cells	Amoeboid, fragmenting
Jordan.....	1916	Pig embryo, animal	Yolk sac, various types of blood cells	Giant, lobed, amitotic, fragmenting
.....	1917	.....	.....	.....
.....	1918	.....	.....	.....
.....	1919	.....	.....	.....
Jenkinson.....	1925	Lemur	Foetal connective tissue cells	Amoeboid, lobed
.....	1926	.....	.....	.....
Nakahara.....	1917	Insect larvae	Silk glands	Hypertrophied
.....	1918	Insects	Adipose tissue	Angular, amitotic
Schürhoff.....	1917	Asparagus	Cells, periphery of veins	Degenerating, giant, fusing?
Saguchi.....	1920	Animal	Pancreas, Islets of Langerhans	Irregular, amitotic, canaled?
.....	.....	.....	.....	.....
Bast.....	1921	Animal	Bone cells	Lobed, amitotic
Champy.....	1921	Animal	Amecocytes, spermatocytes, epithelium, muscle, excretory cells	Indented, canaled, amoeboid
Sokoloff.....	1922	Man	Cancer	Irregular, lobed
Vejdovsky.....	1924	Bee	Adipose tissue	Ramifying
.....	.....	.....	Oenocytes	Canaled?
Levine.....	1925	Man	Carcinoma	Hypertrophied, lobulate
Radtke.....	1926	Euphorbia	Nectaries	Lobed, irregular
Halkert.....	1927	Ranunculus, tulip, snowdrop	Bulbs, tubers	Angular, lobed
Kater.....	1927	Snap bean	Cotyledon	Irregular

TABLE II

RELATION OF PARASITIC ENVIRONMENT TO NUCLEAR MORPHOLOGY

AUTHOR	DATE	ANIMAL OR PLANT HOST	PARASITE	ORGAN OR TISSUE AFFECTED	NUCLEAR FORM
Nawaschin...	1899	Cabbage	Plasmodiophora	Roots	Hypertrophied
Toumeze.....	1900	Plants	Myxomycetes	Root excrescences	Abnormal, enlarged, fragmenting
Magnus.....	1900	Neottia nidus avis, Orchis	Mycorrhiza	Roots	Hypertrophied, amoeboid, canaled?
Tischler.....	1901	Circaea	Nematode	Root galls	Lobed, constricting, fragmenting
Shibata.....	1902	Podocarpus	Root fungi	Roots	Amoeboid, canaled?, fragmenting?
Guttenberg..	1905	Adoxa, Capsella, Zea, Alnus	Puccinia, Ustilago, Albugo, Exoascus, Synchytrium	Fungus galls	Lobed, constricted, branching, canaled
Guttenberg..	1909	Anemone, Mercurialis	Synchytrium	Galls	Canaled
Kusano.....	1909	Pueraria, Amphicarpea	Synchytrium	Stem and leaf tubercles	Hypertrophied, deformed
Wolpert.....	1909	Alnus	Mycorrhiza	Roots	Twisted, amoeboid
Arzberger...	1910	Ceanothus, Elaeagnus, Myrica	Mycorrhiza	Root tubercles	Hypertrophied, amoeboid
Blomfield...	1910	Veronica	Sorosphaera	Stem tumors	Enormous
Meyer.....	1910	Thismia, Burmannia	Mycorrhiza	Roots	Hypertrophied
Némec.....	1910	Colcus, Clerodendron, Pulsatilla, Washingtonia	Nematodes	Galls	Amitotic
Bally.....	1911	Taraxacum potato	Synchytrium	Leaves	Lobed, canaled
Osborn.....	1911	Potato	Chrysophlyctis	Tubers	Lobed, canaled
Reynolds...	1912	Viola	Spongospora	Tubers	Lobed, indented, canaled?
Schirchoff...	1912	Podocarpus	Puccinia	Leaves	Deformed, fragmenting
Spratt.....	1912	Podocarpaceae	Infected cells	Infected cells	Amoeboid
Tobler.....	1913	Anemone	Pseudomonas	Root nodules	Amoeboid amitotic
Edson.....	1915	Beta vulgaris	Synchytrium	Root cells	Canaled, lobed, fragmenting
Kunkel.....	1915	Potato	Phoma betae	Seedlings	Dumb-bell, budding, amitotic
Spratt.....	1919	Leguminosae	Spongospora	Tuber	Lobed, distorted
Allen.....	1920	Club wheat	Root bacillus	Root nodules	Amoeboid, amitotic
			Puccinia	Infected cells	Lobed, dumb-bell, amoeboid

## I. Nuclear form

The studies of nuclear form in lilies and other plants have led me to adopt the following terms in describing the various types of nuclear form:

1. **NORMAL.**—The normal nucleus is ordinarily rounded or oval, with a smooth nuclear membrane inclosing the chromatin granules which lie in the linin network (fig. 1a).

2. **RIDGED OR IRREGULAR.**—The form of the nucleus may be influenced by mechanical pressure of cell inclusions upon it. The nuclear membrane may become irregular in outline because of the

pressure of starch grains upon it, as in the starch storage cells of lily bulb scales (fig. 1*b*).

3. CLEFT.—Lily nuclei very often show a single sharp indentation or deep furrow. Such cleft nuclei are very common in the parenchyma cells of lily leaves, petioles, and stem (fig. 2*a*).

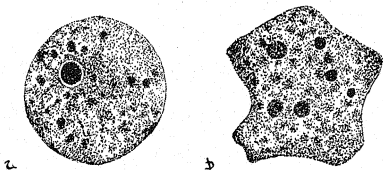


FIG. 1.—*a*, normal nucleus; *b*, ridged or irregular nucleus in storage cell of lily bulblet scale.

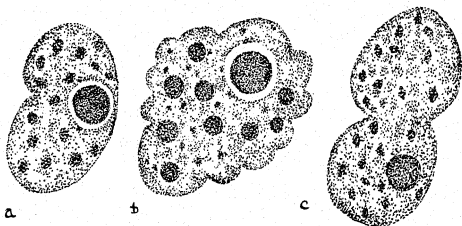


FIG. 2.—*a*, cleft nucleus in lily leaf; *b*, crenate lily nucleus; *c*, constricted lily nucleus.

4. CRENATE.—The crenate form of nucleus is the result of sharp and shallow reticulated furrowing, appearing over the entire nucleus, giving it a nodular surface like that of an orange, but with the elevations larger and more irregular. Such nuclei have been observed in the parenchyma cells of healthy green lily leaves, and in the ground meristem or parenchyma tissue of the flower stems of growing hyacinth bulbs (fig. 2*b*).

5. CONSTRICTED.—The constricted nucleus appears more or less as if it were dividing. Such nuclei occur in the parenchyma cells of

healthy green lily leaves and petioles, and are not an indication of amitosis (fig. 2c).

6. SPINOUS.—The spinous nucleus shows sharp-pointed extensions of the membrane over its entire surface. These nuclei usually occur in regions where material is being transferred from tissues in which starch is being digested to tissues in which cell division and differentiation are going on. They occur in lily bulblets, in cells at the base of scales about the point of origin of young roots, and in lily stems below the growing point and leaf primordia. In long hyacinth roots they are found commonly in the ground tissue. In other

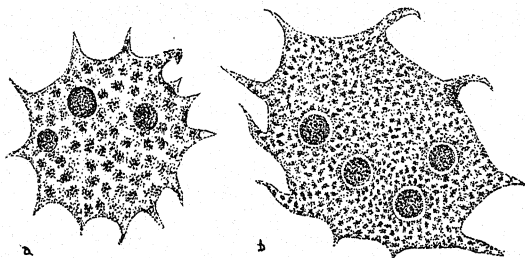


FIG. 3.—a, spinous lily nucleus; b, spinous hyacinth nucleus

parts of the hyacinth plant they are found in parenchyma cells, by the side of the vascular bundles. KORSCHOLT (26) figures a toothed nucleus in a salivary gland of *Chironomus*, in which he observed in living cells that the toothlike processes were put out and withdrawn during the secretory activity of the cells. He believes that there is a gradual passing over through these points of the karyoplasm into the cytoplasm (fig. 3a, b).

7. BULLATE.—Bullate nuclei show extensions of the nuclear mass in the form of rather coarse blunt projections over their entire surface. They are found in the roots of young lily bulblets, in the parenchyma cells of the mesocotyl of the oat seedling, in the parenchyma cells of the leaves on young sprouting narcissus bulbs, and in the starch storage cells of hyacinth bulbs (fig. 4a).

8. **BIFURCATE.**—The bifurcate nucleus is an elongated one which is deeply indented at each end. These nuclei are found most commonly in elongated conducting cells bordering xylem vessels and tracheids in lily bulb scales, bulblet scales, stems, etc., in narcissus bulb scales, and hyacinth bulb stems (fig. 4*b*).

9. **HORNED.**—The horned nucleus shows extensions like those of the bullate nuclei, but larger and more irregular. They are found in lily bulblets, and in the growing points below leaf and branch primordia. In lily and hyacinth roots, large round meristem cells above the growing points contain nuclei which, instead of bearing

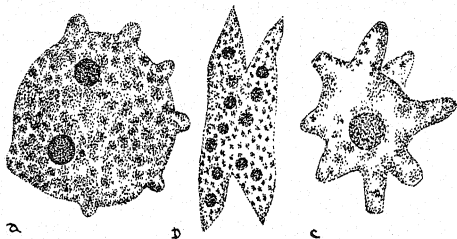


FIG. 4.—*a*, bullate lily nucleus; *b*, bifurcate lily nucleus; *c*, horned nucleus in epidermal cell of buckwheat endosperm.

fine teeth, may show tapering coarser projections or horns (fig. 4*c*). In the old bulb scales of hyacinths, after nearly all the storage starch has been removed, the nuclei appear collapsed, hypertrophied, and show one or several such hornlike projections.

10. **FURROWED OR CANALED.**—So-called canaled nuclei are not really perforated by tubular canals running directly through the nuclear substance. The canals referred to by various writers are simply furrows (seen in section) through the nuclear mass formed by the folding in of the nuclear membrane. This form of nucleus is undoubtedly associated with secretory activity. Such nuclei were found in the cells of bulb scales of the lily and hyacinth when starch was being digested, in the cells of the cotyledons of germinating

peas, soy beans, and bush beans, in the carpellary cells of the iceberg blackberry, in the parenchyma cells of sprouting dahlia tubers in

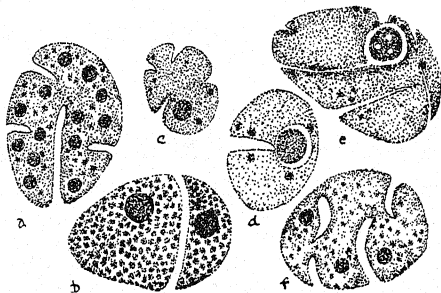


FIG. 5.—*a*, furrowed lily nucleus; *b*, furrowed nucleus in dahlia tuber; *c*, furrowed nucleus in endosperm of buckwheat; *d*, furrowed nucleus in corn endosperm; *e*, furrowed nucleus in bush bean cotyledon; *f*, furrowed nucleus in hyacinth root.

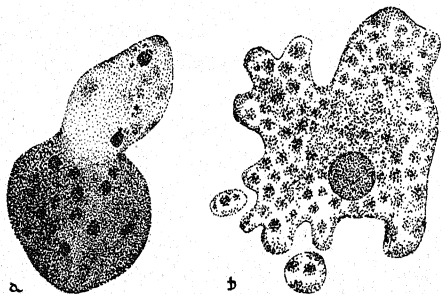


FIG. 6.—*a*, amoeboid nucleus in yellowing lily leaf; *b*, lobulate nucleus in starch parenchyma cell of yellowed lily leaf.

the endosperm cells of sprouting corn and buckwheat, and in the mesocotyl cells of the oat seedling (fig. 5*a-f*).

11. AMOEBOID.—The amoeboid nucleus suggests by its form that there has been some thrusting out of pseudopod-like extensions associated with a flowing movement of the karyoplasm. It has usually been applied to a lobed nucleus which resembles an amoeba with pseudopodial extensions. KORSCHOLT's observations (26) perhaps warrant such an assumption. It may very well be that all the various forms of nuclei if observed in living condition would be found to be changing their forms in a fashion more or less amoeba-like (fig. 6a).

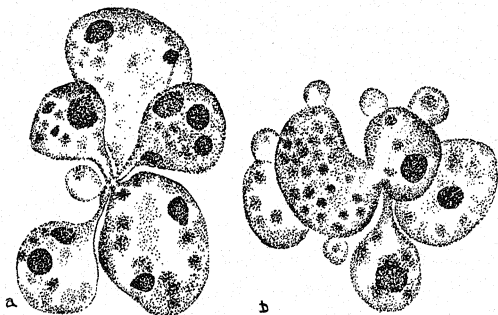


FIG. 7.—Lobed nuclei in starch parenchyma cells of yellowing lily leaves

12. LOBULATE.—The lobulate nucleus shows wide, rounded extensions over more or less of its surface. Fig. 8a shows a nucleus of a blackberry carpel that is furrowed, while in fig. 8b the nucleus appears lobulate. In fig. 8c the lobulate nucleus in a hyacinth flower stem also indicates that it derived its lobulate form from a widening out of narrower furrows. Lobulate nuclei, like furrowed nuclei, are found in regions of digestion, and may represent a higher stage of digestive activity than do the furrowed nuclei. Lobulate nuclei are found in the starch storage cells of lily bulb scales after growth has started, in the starch sheath about the veins of lily leaves, in hyacinth bulb scales, carpels of the iceberg blackberry, storage

cells of pea cotyledons, and in hypocotyls after growth has started (fig. 6b).

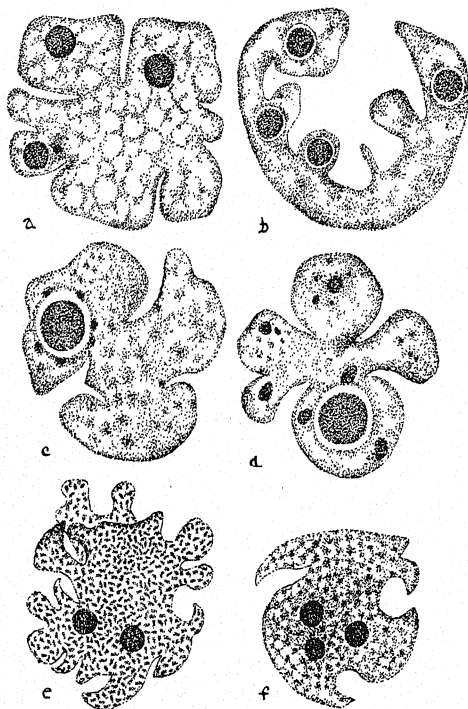


FIG. 8.—a, canaled nucleus; b, lobulate nucleus (cells of iceberg blackberry carpels, courtesy of B. O. DODGE); c, canaled nucleus; d, lobed nucleus (cells of pea cotyledons); e, lobulate nucleus in hyacinth flower stem; f, furrowed (lobed?) nucleus in narcissus leaf.



13. LOBED.—I have distinguished a still more exaggerated type of amoeboid form as typically lobed. In such cases only slender connections unite the lobes, which are thus almost distinct nuclear fragments. This lobed effect may be brought about by a simple widening and deepening of the grooves in a furrowed nucleus. Such a nucleus is almost broken up into a number of separate masses of nuclear material. Lobed nuclei are associated with the same activities as are furrowed and lobulate nuclei, and they occur in the same tissues (fig. 7*a*, *b*).

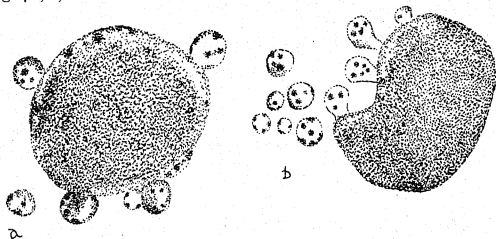


FIG. 9.—*a*, *b*, budding nuclei in parenchyma cells of mottled lily leaf

14. FRAGMENTING, AMITOTIC, AND BUDDING NUCLEI.—Lobing may lead to a final breaking up of the nuclear body into two (or perhaps more or less) rounded fragments. In thin sections the cell may appear to have several or many nuclei, merely because the attachments between the parts of a lobed nucleus are not included in the section (fig. 10*g*). Fig. 10*f* shows a single rounded large nucleus with two smaller rounded nuclei near it. Figs. 9*a* and 9*b* show many minute nuclei apparently budded off from a large rounded nucleus. Such cases of budding nuclei were only observed in lily material.

All these types of nuclei are to be regarded as normal, and specialized in connection with some particular metabolic activity. A later paper will describe certain appearances associated with nuclear degeneration.

## II. Nuclear form in cells of diseased lily plants

### I. NUCLEAR FORM IN CELLS OF BULBLETS OF DISEASED LILIES.—

The bulblets studied were found attached to the mother bulbs of

lily plants that were either stunted in growth, or whose leaves were completely yellowed or blotched, suggesting a virus infection. The bulblets were growing and possessed several young roots. The forms of nuclei found in their various tissues were in general similar to

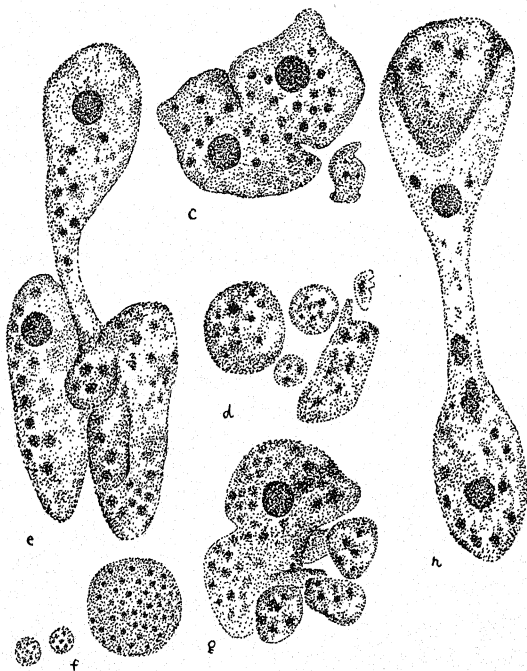


FIG. 10.—*c*, constricted nucleus and nuclear fragment in cell of lily leaf; *d*, group of irregular lily nuclei after fragmentation of hypertrophied nucleus; *e*, lobulate nucleus in xylem parenchyma cell of lily stem; *f*, large rounded nucleus and two smaller nuclei appearing together in lily leaf cell; *g*, lobed nucleus appearing fragmented because of sectioning; *h*, elongated nucleus in xylem parenchyma cell of diseased lily stem.

those described for the cells of healthy bulblets. In elongating cells, destined to become vascular elements, the nuclei appeared to be degenerating, and these cells also contained various kinds of degeneration or secretion deposits. I shall give elsewhere the results of a cytological study of the histogenesis of these vascular elements, with reference to the origin and development of various inclusions (slime bodies, spiral and spindle-shaped structures, wax plates, etc.) which occur in such tissues.

2. NUCLEI IN APPARENTLY HEALTHY LILY PLANTS.—Material was studied from large, apparently healthy bulbs of *Lilium longiflorum*, *L. giganteum*, and *L. harrisii*, which were purchased from commercial seed companies. Certain parts of such bulbs were fixed and sectioned immediately after removal from storage, other parts later on after growth had begun. This material shows clearly that irregularity in nuclear form is associated with increased activity in the plant. In the case of the resting bulbs, the nuclei in the thick outer scales, thinner inner scales, bulb stems, and growing points were found to be rounded in form, and normal in appearance and size. Moderately toothed forms occur in the pith and cortical parenchyma cells of the stem, which contain small grains of storage starch. In the elongated conducting cells spindle-shaped and bifurcate nuclei are found.

In bulbs that have started growth, lobed nuclei are found in the storage parenchyma of the outermost scales, where starch digestion is taking place. In elongated conducting cells below the growing points, the nuclei appear fragmenting by a pinching off of small rounded nuclei. Many cleft nuclei are found in the parenchymatous cells of the young leaves. In the young roots the nuclei are normal excepting near the growing point of the root, where they show minute toothlike projections.

The nuclei of the palisade and parenchyma cells of the leaves are generally normal in appearance, and may be slightly indented or cleft. Some of the nuclei may even be lobed. In the cells of senescent leaves from the base of otherwise healthy lily plants, the nuclei may be lobed and irregular in the spongy parenchyma cells. Many cells appear binucleate. Some of these cells contain grayish deposits or secretion bodies of an apparently oily nature. These bodies can be distinguished from the  $\alpha$ -bodies of tobacco mosaic by the irregularity of their outlines.

3. NUCLEAR FORMS IN CELLS OF MOSAICED LEAVES.—Many of the lily plants show unquestionable symptoms of mosaic, with typical *x*-bodies in the leaf cells (fig. 11). The plants have not been studied from the standpoint of their possibly contain-

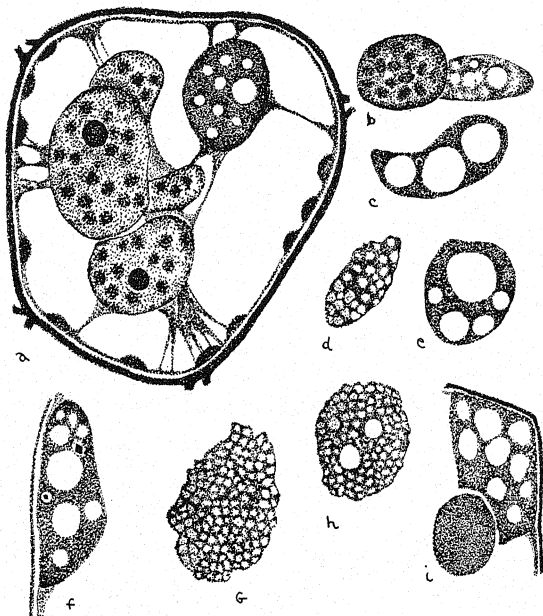


FIG. 11.—*a*, parenchyma cell in mottled leaf containing hypertrophied lobed nucleus and large vacuolate *x*-body; *b*, vacuolate *x*-body attached to normal nucleus; *c*, vacuolate *x*-body; *d*, secretion body; *e*, *x*-body including two red staining granules; *f*, large *x*-body containing two cuboidal red stained protein grains and red stained rounded granule; *g*, *h*, secretion bodies; *i*, vacuolate *x*-body in corner of cell, with dead shrunken red-stained nucleus pressing against it (by focusing, the *x*-body can be seen quite distinct from primordial utricle).

ing a transmissible virus, or as to the general course of the disease. The starch in these diseased leaves is in general found only in parenchyma cells about the vascular bundles. The nuclei in these cells, where digestion of the storage starch is going on, are very irregular in form, and appear hypertrophied, amoeboid, or deeply lobed. These cells often contain small nuclei, which may have been formed by the budding of larger nuclei, or by fragmentation of lobed nuclei. In some of the parenchyma cells there are red staining bodies lying in the cytoplasm that appear to be extrusions of the chromatin or nucleolar material from the nucleus. Many nuclei are markedly irregular in outline, suggesting flowing movements of the karyoplasm. In these leaves the secretion bodies (fig. 11*d*, *g*, *h*) found in senescent leaves also appear. The  $\alpha$ -bodies are unusually large, take the orange stain, and contain several large rounded vacuoles (fig. 11*a*).

In connection with the studies on the cytology of plants affected with virus diseases (10, 11, 12), I have noted the general absence of irregular or hypertrophied nuclear forms. This is in strong contrast with the general situation as to plants infected with intracellular parasites. This leads one to believe that the irregular and hypertrophied nuclei in mottled and yellowing lily leaves are not due to the presence of the virus but to the dying condition of the leaves.

4. NUCLEI IN DISEASED GROWING POINTS AND STEMS.—In the growing points of plants which appear diseased and stunted in growth, exceedingly hypertrophied nuclei are found in the ground meristem and parenchyma cells about and between the vascular bundles. In these cells also occur grains of storage starch, which show evidence of digestion in their irregular outline, and possibly products of digestion in the form of orange and red staining granules. Huge twisted nuclei or lobed forms occur in the xylem parenchyma cells, and the elongated conducting cells contain bifurcate nuclei. Large  $\alpha$ -bodies occur commonly in the parenchyma cells about the veins. Grayish, apparently oily secretion deposits are also present in these cells.

### Conclusion

These cytological studies of plants infected with virus diseases impress one with the fact that the nucleus is hardly affected structurally by the diseased condition. At the most it has been found in

tobacco that the disease is associated with a slight increase in size, and a very slight irregularity in outline of the nucleus. Tobacco and dahlia mosaic tend rather to be inhibitive and destructive to the cell elements as well as to the plants. Many intracellular parasites, such as the chytrids, induce hypertrophies and hyperplasias. The virus diseases tend rather to stunt the development of the cell and of the entire organism, even killing the cells in some cases, although in general mosaiced plants are able to reach maturity and set seed.

The very numerous and striking cases of nuclear deformation in specialized tissues in the lily are at least not obviously associated in any way with the presence of the  $x$ -bodies. The nucleus is the center of all physiological activity of the cell, and becomes during periods of very active and special phases of metabolism exceedingly hypertrophied and irregular in form. Such hypertrophied nuclei with cleft, crenate, or furrowed membranes, or lobed, lobulate, or amoeboid outlines, occur normally in healthy plants, in special organs of nutrition, and associated with certain phases of nutritional and secretory activity.

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## PLANT SUCCESSION AND ECOLOGICAL HISTORY OF A CENTRAL INDIANA SWAMP

STANLEY A. CAIN

(WITH EIGHT FIGURES)

Bacon's Swamp, with an approximate area of 30 acres, lies in the E 1/2, SE 1/4 of Section 6, Washington Township, Marion County, Indiana. A rather narrow arm of the swamp runs north and south. The southern end of the swamp swings southwest and broadens out to somewhat more than a quarter of a mile in width. This retort-shaped basin is obviously of glacial origin, as it is entirely inclosed by slopes exposing glacial material. In general the upland is level or slightly undulating. There are a few places in the vicinity with moderate morainal topography. Glacial boulders occur at the surface in relatively few places. Bacon's Swamp is a unique feature of the vicinity, which is without lakes or swamp except for two very small swampy areas, one a mile away and the other half a mile distant. There are, of course, swampy places along the flood-plains of the streams. The basin in which Bacon's Swamp lies is a constructional feature of the Bloomington Ice Sheet of Early Wisconsin glaciation. MALOTT (7) shows a morainal region in the middle eastern part of the county. Bacon's Swamp should probably be assigned to this feature.

According to GEIB and SCHROEDER (6), Marion County includes three distinct geological formations: Carboniferous limestone in the eastern part of the county, which is underlain by black Genesee shale in the west, and Knob sandstone in the southwest. These rock formations, however, are covered to a depth of 50-100 feet by a deposit of drift which forms the surface of the county and determines the character of the soils. The soils of Marion County belong to the Miami and Huntington series. The Miami soils have been derived directly from the glacial material which covers the whole region. The Huntington soils are alluvial. Bacon's Swamp lies in the Miami clay loam, which is by far the most extensive soil, oc-

cupying 76 per cent of the county. The Miami black clay loam occupies only a small percentage of the county, and occurs in small basin-like depressions throughout the upland.

The material composing the Miami black clay loam is of glacial origin, but since its deposition it has been modified to a considerable extent. Prior to the construction of drainage systems sufficient to carry off the surplus water, these small areas were covered with swamps and marshes. Through the growth and decay of vegetation large amounts of organic matter have been added to the soil, the rapid oxidation of which was prevented by excessive moisture. Some of the finer particles of soil have been washed into these depressions from the surrounding upland, and this has had considerable influence on the texture of the soil.

Only two areas of muck are mapped in the survey of Marion County, and these are very limited. The material consists of vegetable matter in advanced stages of decomposition, with fine earth from the higher surrounding land intermixed. The muck is black, and extends to a depth of from 18 inches to 4 or 5 feet, usually resting upon a bed of clay. There is no mention in the soil survey of any peat deposits in the county. Evidently the presence of Bacon's Swamp was unknown to these men, since, as has been stated, the swamp is a unique feature of the area.

The basin in which the present swamp lies is lined with a fine compact blue silt which has been washed in from the surrounding soil. Borings around the edge of the swamp have reached this practically impenetrable layer at a depth in places of only a few inches. Sometime ago Mr. DOUGLASS (5), of Trevlac, Indiana, made borings through the peat in the center of the swamp. In recent correspondence with the writer he stated:

I prospected Bacon's Swamp for the peat and invented a machine for compressing it for fuel purposes, back about 1905. My boring outfit had only 35 feet of pipe and I failed to touch bottom at the deepest place, all solid peat. The entire swamp is underlaid with a heavy, water-retaining, blue clay. I have no idea how thick this layer may be but it serves to hold the water in the swamp as perfectly as though it were a crockery bowl.

The final recession of the Bloomington ice sheet (perhaps 60,000 years ago) no doubt left a lake at the present site of Bacon's Swamp.

From the time the last ice left this latitude, and the small post-glacial lake was formed, the vegetational history has been in progress. As stated by BLATCHLEY (1):

A lake of small size begins to die the moment it is born. Its basin begins to fill with material other than water, and the process of final extinction is commenced. There are more beds of extinct lakes in northern Indiana today than there are existing ones. These former basins are now sites of bogs or meadows underlain by 15 to 29 or more feet of muck and marl.

Bacon's Lake is now extinct. The margin is grown up with water-inhabiting trees and the interior is occupied by a wet meadow. The vegetation of Bacon's Swamp presents some intriguing aspects. An understanding of the relationships of the various plant associations depends upon a knowledge of the past vegetational history of the area. It would be better, perhaps, to describe the vegetation as it now exists and then proceed to certain evidences of the earlier vegetational conditions.

#### Present vegetation

The center of the swamp is occupied by a wet meadow ordinarily inundated from 8 to 15 inches, and dominated by *Calamagrostis canadensis*<sup>\*</sup> with an abundance of *Dulichium arundinaceum*, *Juncus canadensis*, *Aspidium thelypteris*, and *Hypericum virginicum*. There are two places where this meadow is interrupted. The first is a rectangular area of open water near the center of the swamp where it bends to the southwest (figs. 1, 2). This pond is the result of an attempt to construct a road across the swamp. In the fall of 1914 the township built a dirt and gravel road, now known as 56th Street, from College Avenue on the west to Keystone Avenue on the east. The following winter the portion of the road bed constructed on the peat across the wet meadow of the swamp disappeared, the weight of the road bed compressing the loose water-soaked peat.

The vegetation about this open water would be quite puzzling if the origin of the pond were not known. The plants of the wet meadow grow almost to the edge of the open water, from which there is a very abrupt transition. The normal series, from submerged hydrophytes through rooted hydrophytes with floating leaves to

<sup>\*</sup> The nomenclature is that of GRAY's *New Manual of Botany*, 7th ed.

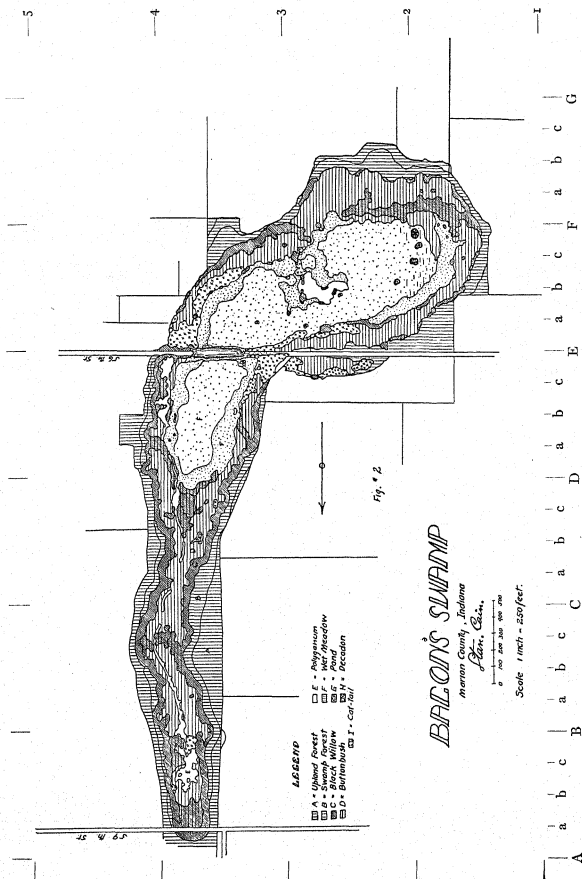


FIG. 1.—Vegetation map of Bacon's Swamp derived from airplane photographs (2): letters and figures at sides of map facilitate location of photographs and references in connection with this study; for dimensions apply the reduced scale of map itself.

erect hydrophytes, as *Typha*, etc., is not represented here. The absence of these stages is explained by the abruptness with which the substratum drops off from water about a foot or less in depth, where the typical meadow is found, to a depth too great for *Nymphaea*, etc., within a horizontal distance of 3 or 4 feet. However, a few scattered plants of *Cephalanthus* are making their appearance

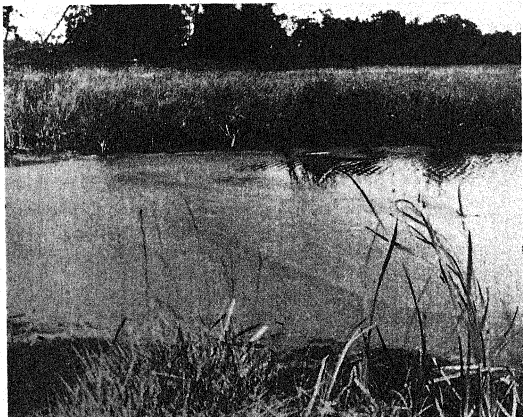


FIG. 2.—(Ea-3.5, N)\* The 56th Street pond, showing abruptness with which *Calamagrostis* meadow stops, eliminating all stages from meadow to open water, except for scattered specimens of *Cephalanthus* which are just appearing.

\*The parenthetical numbers and letters in this and following figure legends indicate exact location and direction of photograph in map (fig. 1); for example, N (north) indicates direction in which camera was pointed.

along the north side (fig. 2). In addition to the buttonbush, a few scattered species, as *Polygonum hydropiperoides* and *Scirpus cyperinus*, are to be found. As the area of the open water is not great there is practically no wave action, and also there is little inwash of material, except at the ends of the pond, as the peat is fibrous and bound by roots; thus the abruptness of the sides of the depression is very slowly changing.

The other region with open water is found in about the center of the wide southwest end of the meadow, and is connected with the moat on the south by a growth of *Typha*, *Polygonum*, *Carex*, etc., occupying water too deep for the plants of the ordinary meadow. This region is of quite a different type from the one just described. In the first place it is entirely natural and unaffected by man. It lies over what is probably the deepest part of the basin (over 35 feet deep), and is the last to be filled by vegetational deposition. The substratum is very light and spongy, so much so that it cannot be traversed. The first plants to appear around the open water are *Polygonum hydropiperoides* and *P. muhlenbergii*, rooted in the muck. *Nymphaea advena*, *Sagittaria latifolia*, *Ranunculus delphinifolius*, etc., do not appear here as they would under similar conditions elsewhere. The explanation for this is probably revealed by studies of the hydrogen-ion concentration (CAIN 3). This region is about 100 times as acid as is the region previously discussed, or as is the usual habitat of these plants.

Near this hole occur clumps of shrubs, as *Salix discolor* and its variety *eriocephala*, *S. sericea*, and *Cephalanthus occidentalis*. It is a significant feature of this region that scattered patches of *Decodon verticillatus* occur along the edge meadow-mat next to the open water (fig. 3). This fact, together with the presence of *Sphagnum* remnants in the peat, indicate typical bog conditions sometime in the past.

Scattered over the swamp in a number of places are hydrophytic associations which are typical for drained swamps. These regions, which are to be found mostly between the meadow and the button-bush, have the ordinary freshwater succession. Usually the first stage of submerged aquatics is poorly represented, probably because of the peaty nature of the substratum. For example, *Ceratophyllum*, *Myriophyllum*, *Vallisneria*, etc., seem to be entirely absent. This stage is here represented practically entirely by algae and diatoms, and various microhydrophytes.

The second stage, composed of rooted hydrophytes with floating leaves, is characterized by *Nymphaea advena*. Another plant which should be placed here is *Ranunculus delphinifolius*. A later development in the succession is that of the erect hydrophytes, rooted in the

muck and raising their leaves above the water. The species of *Polygonum* seem to appear first in this stage. There are three species occurring at Bacon's Swamp, *Polygonum hydropiperoides*, *P. muhlenbergii*, and *P. sagittatum*. *Sagittaria latifolia* is a very common member of this group. *Typha latifolia* seems to occur later, and tends to replace the previous plants. The explanation of this

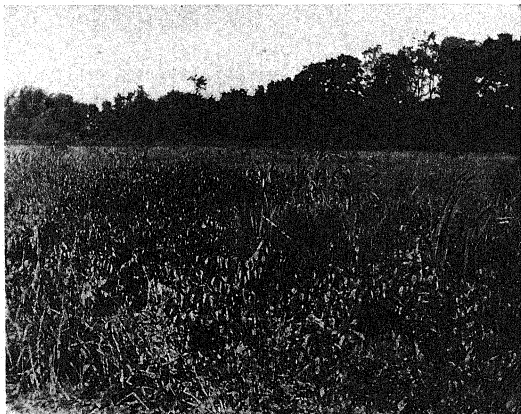


FIG. 3.—(Eb-2.7, NW) An "island" of *Decodon* near edge of open water in southwest lobe of swamp.

immediate succession probably lies in the shading of the broader leaves of the lower-growing plants by those of the cat-tail, which rise to considerable height. Thus the broader-leaved and lower-growing plants suffer, in their turn, the fate they meted out to the plants with floating leaves which preceded them. The other important factor is that of the depth of water, each stage filling in with its own detritus until the depth will permit the growth of the succeeding stage. The matter of shading, however, within limits of depth, seems to be the controlling factor. *Typha* is found growing



in water from 1 to perhaps 2 feet deep. At Bacon's Swamp *Typha* seems to occupy a rather unusual position, between the hydrophytes just described on the one hand and the wet meadow on the other, while in places it is intermediate between the wet meadow and the buttonbush. Of the two, the buttonbush enjoys a wider distribution than the cat-tail; growing in deeper water, it forms a practically continuous zone about the wet meadow inside the willow zone (fig. 1).

There is a very interesting group of small floating plants deserving special mention, including *Lemna minor*, *L. trisulca*, *Riccia fluitans*, and *Wolffia columbiana*. The different members of this group can be found almost anywhere in the swamp (that is, in the meadow, the buttonbush zone, or the ponds) varying in relative abundance from one season to the other. Fig. 2 shows the surface of the 56th Street pond practically covered with *Wolffia*.

The wet meadow, which occupies the whole center of the swamp, with the exceptions previously noted, grows on the highest part of the peat. For the last 2 years the center of the meadow has been covered with 8 inches or more of water. During previous drier seasons the water has been reduced to below the surface of the peat for part of the year. During the drier periods fires have sometimes occurred, producing considerable holes in the surface layers of the peat; however, submergence seems to be the usual condition of the meadow. Toward the outer edge the water becomes deeper. At about 12-15 inches the dominant *Calamagrostis canadensis* and its associates give way to *Carex impressa*. The sedge flourishes to a depth where a pure stand of *Cephalanthus occidentalis* is to be found. This stocky shrub grows in water 2-4 feet or more in depth. It is most characteristic for these plants to show an abrupt transition, but in some places they intermingle or *Typha* is intermediate.

From the interior of the swamp one can see quite distinctly the concentric zones of vegetation, each rising somewhat higher than the one next inside it. Standing in the *Calamagrostis* meadow one sees first the *Carex* zone, then successively the buttonbush, black willow, and the swamp and upland forests in the rear. Some of these zones are indicated in figs. 4 and 5.

## Former vegetation

The southwest end of the swamp is the most interesting portion. It is here that the concentric zones of vegetation are broadest, and their relations to each other and their probable historical development are clearest. One unique feature of this region is seen in the "islands" of *Decodon verticillatus* (figs. 3, 6). These islands are rem-



FIG. 4.—(Eb-2, N) View from interior, showing some of concentric zones indicated at margin: (a) *Calamagrostis* meadow, (b) *Cephalanthus* zone, (c) *Salix nigra* zone, (d) swamp forest.

nants of former conditions when Bacon's Swamp was occupied by a typical *Sphagnum* bog association. In the fall of 1927, and again in 1928, a little *Sphagnum* was able to reestablish itself after a dry period, but there had been none for a few years before, and this was not able to maintain itself. The surface peat shows, on microscopic examination, a considerable admixture of *Sphagnum* along with remnants of the typical meadow plants of the present; but the underlying peat can be described only as Mr. DOUGLASS has done, as

"a pure *Sphagnum* peat." At present the *Calamagrostis* meadow comes to the margin of these islands but seems unable to eliminate them, although the water is usually less deep. This is undoubtedly due to their habit of rooting at the tips of the branches, which results in a dense tangle of plants. These descending branch tips show fine development of aerenchyma where they are submerged. The surface



FIG. 5.—(F-2.4, W) Another interior view of swamp, showing extension of black willow into central part; zones indicated thus: (a) *Salix*, (b) *Cephalanthus*, (c) *Carex impressa*.

water in this vicinity is considerably more acid than it is at the other end of the swamp. This is due to the still greater acidity of the underlying peat, which reaches a pH of 4.4. The southwest end of the meadow differs from the rest in another respect. Quantities of *Cephalanthus* are scattered throughout, particularly between the *Decodon* and the *Carex* (fig. 1). A close examination of these clumps of buttonbush shows that these plants have at no distant time experienced better living conditions. Everywhere the shoots rise from

among dead branches of a more luxuriant growth (fig. 7). In all clumps examined the root systems were heavier than the present growth would warrant, and old stems were present. There seems to be only one explanation: for some reason the water level has recently been changed so that *Cephalanthus* is losing out, and the meadow is coming to occupy a greater portion of the area. The same phenomenon probably also accounts for the rapid disappearance of

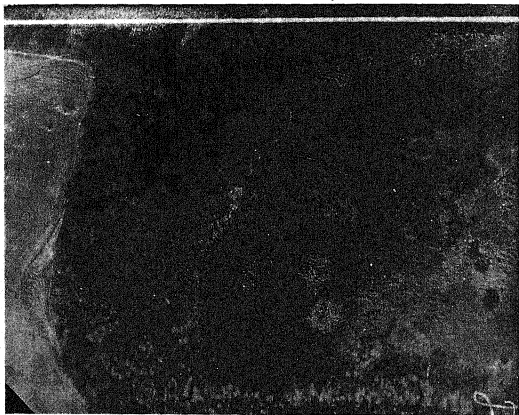


FIG. 6.—(Ea to Fa and 1 to 2.5) Airplane photograph of southwest end of swamp, showing "islands" of *Decodon* conspicuous near center (compare with fig. 1; photographed from altitude of 2000 feet).

*Sphagnum* from the mat. I have studied the conditions of the swamp intensively during the past 4 years, and have not found any living *Sphagnum* except that mentioned. It is very interesting, however, that *Sphagnum* has grown there in abundance until quite recently. Mr. DOUGLASS, who knows the swamp very well, has responded to my inquiries concerning the presence of *Sphagnum* as follows:

It is difficult for me to believe that you now find no *Sphagnum* at Bacon's Swamp. . . . Certainly it grew there as abundantly and richly as I ever saw

it in a Wisconsin or Michigan swamp, even better. In proof of this you will find that the peat of Bacon's Swamp is a true *Sphagnum* peat, one of the purest *Sphagnum* peats I ever examined, and at one time when I was interested in the subject I examined many samples. Enclosed please find a paper prepared for the Indiana Academy of Science many years ago (DOUGLASS, 1905). All the pictures in this paper were taken at Bacon's Swamp. . . . The one showing a block of peat is sufficiently clear to show typical *Sphagnum* moss on the top.

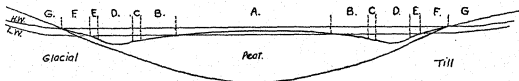


FIG. 7.—(Ec-2) *Cephalanthus* from wet meadow; note dead branches characteristic of buttonbush in this region.

Other inquiries led to a similar statement by Professor C. F. Cox, of the Technical High School, Indianapolis, who said, "I have collected *Sphagnum* at Bacon's Swamp as recently as 1919 and 1920." The fact of the rapid disappearance of *Sphagnum*, together with the more gradual restriction of *Cephalanthus* in the southwestern end of the swamp, leads to the conclusion that the water table, in the immediate vicinity at least, has been lowered in the past decade or two. As the region has been practically deforested and under agriculture for almost a century, these factors cannot be used to

explain the recent changes. They are probably due to tilling. The lowering of the water table in the vicinity would affect the drainage of the surface peat in the central meadow. This would hasten the disappearance of *Sphagnum* and the restriction of *Cephalanthus*, and at the same time favor the spread of the *Calamagrostis* meadow.

From a consideration of the depth of the water in the different associations, it is already apparent that, for a swamp, the substratum has an unusual topography (fig. 8). The different depths of the water are intimately related to the plant associations, and become a limiting factor, at least in some cases. The convex surface of the peat, with the greatest height in the center, together with the moat at the periphery, is a further suggestion of the dual nature of the



Vertical Sector across Bacon's Swamp [Vertical scale exaggerated] H.W. = High water, L.W. = Low water. The concentric associations indicated are as follows: A-*Calamagrostis* meadow, B-*Carex* meadow, C-*Typha*, D-*Cephalanthus* (moat), E-*Salix nigra*, F-*Swamp forest*, G-*Upland forest*.

FIG. 8.—Relation of the various swamp associations to water level, high and low; vertical scale greatly exaggerated.

area. It is quite common for even the most typical bog formations to be surrounded by a zone of fresh water plants. Many times in small lakes the bog mat appears, not over the shallower littoral portions of the water, but out from the shore where the water is deeper. So it is that an old lake when practically filled with vegetation, may have a greater accumulation of peat in the center (where it is built under bog conditions of high acidity and poor drainage) than at the periphery, where oxidation and drainage are greater, and the different types of vegetation build more slowly. This would seem to be another evidence of the former acid bog vegetation at Bacon's Swamp. It is likely that the regions now occupied by *Cephalanthus*, *Salix*, and the swamp forest have been under swamp conditions since the first. The earlier stages of the swamp succession, of which the woody plants represent the culmination, have long since been

eliminated by the presence of the bog mat in the main body of the water.

TRANSEAU (8) notes the fact that the succession from open water to the higher bog margin can be traced easily, but that throughout the region of northern Indiana and Ohio and southern Michigan there seems to be no connection between this succession and the plants of the bordering forests. He states:

The zonal succession of the plant groups, from the submerged aquatics of the pond to the arborescent forms of the higher bog margin, is clearly defined and well known. But then comes a sudden break, and without a suggestion of gradation the surrounding forest appears.

Since Bacon's Swamp is out of the present range of distribution for most of the common bog trees and shrubs, they would not be expected there within recent times; yet there is little doubt that boreal forms persisted at the swamp long after the main wave of the northern migration had passed by, as they are now found in bogs farther north. Also, this need not interfere with the situation being somewhat as TRANSEAU has described for northern Indiana, for there seems to be even less connection between the meadow and the buttonbush-swamp forest-upland forest succession around the periphery of the depression. This region has probably been under swamp conditions for a very long time, whereas the central part, now occupied by the *Calamagrostis* meadow, has probably been under bog conditions until recently. This fact would account for the apparent hiatus between the succession to the meadow and the succession from the moat to the upland beech-maple climax forest.

COWLES (4) speaks of the marginal flora which is to be found about most types:

All of the peat bogs have a characteristic marginal flora, i.e., the vegetation at the margin of the original lake is essentially alike in all cases. These plants, as well as those of the *Cassandra* bogs, are the same over wide areas. The most common species of the bog margin flora are *Nyssa sylvatica*, *Populus tremuloides*, *Ilex verticillata*, *Pyrus arbutifolia*, *Spiraea salicifolia*, *S. tomentosa*, *Rubus hispidus*, *Gaultheria procumbens*, *Osmunda cinnamomea*, *O. claytoniana*, *O. regalis*, *Betula papyrifera*, and *Polytrichum commune*. This vegetation originates outside of the swamp, and may be regarded as xerophytic; however, it often encroaches upon the swamp as the latter develops.

It should be said that more than half of the plants just mentioned are found in the flora of Bacon's Swamp, and in general the description fits fairly well with the common marginal flora and development found throughout the region.

Bacon's Swamp is considerably farther south than the present limits of bog distribution (TRANSEAU 8). That Bacon's Swamp, like a number of similar areas outside the present distribution of bogs, has passed a part of its earlier existence and vegetational history under bog conditions is to be expected in connection with the northward trend of vegetation following glaciation. The present study is unique in that the transition from bog to swamp has been so recent, and has been under a degree of scientific observation during the time. The depth of the basin compared with its surface area has no doubt had some influence on the long duration of the acid bog conditions, so that an unusually deep deposit of peat is to be found there.

#### Peripheral vegetation

Occupying what I have called the moat is a zone of *Cephalanthus occidentalis*, ranging up to 200 feet in thickness. For the most part this species exists alone, but in places it contains an admixture of *Ilex verticillata*. The *Ilex* nearly always occurs next to the swamp forest and not on the side next to the wet meadow. There is one particularly interesting shrub area on the west side of the swamp (fig. 1, E-2.5 to 2.8). Some buttonbush is to be found there, but it does not characterize the vegetation. Among the other woody plants are *Xanthoxylum americanum*, *Ilex verticillata*, *Aronia floribunda*, *Salix discolor*, *S. discolor* var. *eriocephala*, *S. sericea*, *Rosa palustris*, *Cornus amomum*, *Rubus hispidus*; and a few trees, principally *Acer rubrum* and *Populus deltoides*. With these woody plants may occur quantities of *Aspidium thelypteris*, *Polygonum sagittatum*, *Scirpus cyperinus*, and *Typha latifolia*. In regions where the shrubs are absent and there is not a great amount of shade a typical fen is found, with numerous species, among which are *Lobelia cardinalis*, *L. siphilitica*, *Asclepias incarnata*, *Eupatorium perfoliatum*, *Lycopus uniflorus*, *Apios tuberosa*, *Penthorum sedoides*, *Bidens trichosperma*, and *Saururus cernuus*. There is no other place in the swamp where



there is such an association of plants. An examination of the substratum shows a considerable admixture of sandy soil washed in from the adjacent upland. This region is also subject to considerable fluctuation in the water level, and ranges around the neutral point in acidity.

The swamp forest, which everywhere surrounds the swamp outside the black willow zone, is the last of the zones to be directly connected with the swamp (figs. 1, 8). If *Salix nigra* is considered as a part of the swamp forest, it must be clear that this single species forms a continuous, although narrow zone between the buttonbush and the rest of the forest. In places the willow may even penetrate the buttonbush zone for considerable distances, as along the southern side of the western end of the swamp (figs. 1, 5). The buttonbush does not grow well under the willow, probably because of the shading. In so far as the depth of the water is concerned, neither seems to have an advantage over the other. It would seem from the peripheral location of the willow that it requires a greater admixture of soil with the peat.

The swamp forest proper is made up of *Acer rubrum*, *Ulmus fulva*, *Nyssa sylvatica*, *Fraxinus profunda*, *F. nigra*, *Quercus bicolor*, *Q. palustris*, and *Populus deltoides*. The red maple is to be found throughout the whole region, as is also the elm. Along the north end of the swamp are some pure stands of *Fraxinus profunda*. *Q. bicolor* and *Nyssa* are not found in any quantity. The ordinary woodland herbs are missing from this association, due to the long periods of inundation each season. Along the upper margin of the swamp forest occur *Onoclea sensibilis*, *Carex crinita*, *Ranunculus recurvatus*, *Saururus cernuus*, and *Impatiens biflora*. The upper limits of the swamp forest are the upper limits of inundation (fig. 8). The swamp forest gives way to the upland beech-maple forest which is the climax for the region. Although there is no virgin timber, there are trees large enough to give a good idea of the luxuriance to which this forest once attained.

My lists for the fragments of woodland surrounding the swamp include most of the characteristic species of the upland climax. There are 23 species of trees, including the beech, white and black sugar maple, tulip, poplar, wild black cherry, five species of ash,

four of oak, two of hickory, the Kentucky coffee tree, honey locust, and others. There are 11 species of low trees, forming a second layer in the woods, as follows: two dogwoods, papaw, redbud, blue beech, ironwood, choke cherry, Canadian plum, smooth sumach, and nannyberry. There are seven lianas, including two greenbriers, moonseed, Virginia creeper, poison ivy, trumpet creeper, and summer grape. Besides there are nine species of low shrubs. This gives a total of 50 woody plants in about one-fourth as many acres.

### Summary

1. Bacon's Swamp is now occupied by a group of associations characteristic of half-drained swamp areas of the central west.

2. The vegetation in the past has pertained more to the character of undrained swamps. Evidence of this rests on the following points: (a) the luxuriant growth of *Sphagnum* some 20 odd years ago, and its known presence up to 7 years ago;<sup>2</sup> (b) the presence of quantities of well preserved *Sphagnum* remnants in the surface and deeper layers of the peat, particularly in the deeper southwest lobe of the swamp; (c) the higher acidity of the surface water, which is a result of the even higher acidity of the underlying peat; (d) the topography of the peat, which is more characteristic of the type of filling-in found in bogs than in ordinary lake deposition, in that the higher portion of the peat is in the center of the swamp and diminishes toward the periphery, until there is a moat filled with 4 or 5 feet of water which entirely surrounds the wet meadow.

3. There has been a recent lowering of the water table (some-time within the last quarter of a century) which may be taken to account for: (a) the disappearance of the *Sphagnum*; (b) the restriction of the buttonbush to the moat, that is, the elimination of it from the wet meadow, accompanied by the spread of the *Calamagrostis* group.

4. The climatic conditions in the vicinity are such as not to preclude the presence of bog vegetation.

5. The hydrophytic succession, found particularly in the middle and northern, less acid, parts of the swamp, progresses from (a) submerged aquatics; (b) rooted aquatics with floating leaves; (c)

<sup>2</sup> There has been some germination the past two years but no reestablishment.

floating aquatics; (d) rooted aquatics, first with broad erect leaves and later with taller narrow leaves; to (e) the meadow with *Carex* around the deeper margin, and *Calamagrostis*, etc., in the center. These steps are related consecutively to decreasing water depth, with shading as a factor.

6. From *Typha* there seems to be another sequence not definitely related to the one just described, but which starts with the moat and works outward. It consists of (a) *Typha*, (b) *Cephalanthus*, (c) *Salix*, (d) swamp forest, and (e) upland climax forest. The early stages of this sequence seem to have been eliminated by the encroachment of the vegetation of the peat mat in the center. The region of these outer zones has probably never shared in any of the bog history of the central part of the swamp.

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# NUCLEOLUS IN ROOT TIP MITOSIS IN ZEA MAYS

CONWAY ZIRKLE\*

(WITH PLATES XII, XIII)

## Introduction

In spite of the fact that the nucleolus is in many ways the most conspicuous object in the cell, and has been the subject of numerous investigations during the last 150 years, our knowledge of its composition, function, and behavior during mitosis is most fragmentary. There is little agreement among cytologists who have studied it concerning any phase of its life history. Among the chief points of dispute have been whether or not it was composed of chromatin, and whether or not it contributed to the karyokinetic figure or fragmented and passed out into the cytoplasm. If the nucleolus were in reality two different cell organs, so alike morphologically as to be frequently confounded with each other, there would be a ready explanation for the present confusion. WILSON (16) has indeed provisionally classified the nucleoli into two groups: (1) plasmosomes, or true nucleoli; and (2) karyosomes, or chromatin nucleoli.

Aside from the further complication of the issue by certain workers mistaking clumps of badly fixed chromatin for nucleoli, as WALKER and TOZER (15) have pointed out, undoubted nucleoli have been reported to be composed of chromatin, while others have been recorded as containing no chromatin whatever. There is, of course, no reason for assuming the nucleolus to be composed of the same substances throughout the entire animal and plant kingdoms, yet the evidence for the existence of two distinct types would be more convincing if the same nucleolus had not frequently been reported by different investigators as belonging to each category.

The rôle which has been assigned the nucleolus in the economy of the cell has been quite varied. LUDFORD (6), WILSON (16), and SHARP (12) have summarized these divergent views, so no complete or general account of them will be given here. It may not be amiss,

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however, to list a few of them. It has been held that: (1) the nucleolus is an accumulation of waste products of the nucleus, and is periodically fragmented and passed out into the cytoplasm; (2) the nucleolus is the center of the vital activity of the cell and in fragmenting it carries the chromatin out into the cell; (3) the nucleolus carries the excretory and secretory products out from the nucleus to the cytoplasm; (4) the nucleolus originates in the cytoplasm and serves to carry nutrition to the nucleus; (5) the nucleolus conducts the vegetative processes of the cell; (6) the nucleolus controls the somatic functions, metabolism and movement; (7) the nucleolus is a storehouse for the chromatin of the resting nucleus; (8) the nucleolus is a reserve constituent of the linin reticulum; (9) the nucleolus is a storehouse of nucleic acid to be drawn upon as the chromosomes resume their basophilic character; (10) fragments of the nucleolus pass out into the cytoplasm of the egg and give rise to yolk granules; (11) the fragments of the nucleolus become mitochondria; (12) the nucleolar globules are bearers of stimulating or finishing material of the genes; (13) the nucleolus is a reserve product of metabolic activity and is used in the upbuilding of certain parts of the cell; (14) in the pancreas the nucleolar matter elaborates zymogen; and (15) in certain insects the nucleolus contributes to the activity of the silk glands.

The most common method of cytological investigation of the nucleolus has consisted in examinations of sections fixed by the usual methods, in which the nucleolus was distinguished from other cell organs by its reaction to specific stains. Thus its oxyphilic properties were established. It could in certain mitotic phases be distinguished from the chromatin by this method, as it united with acid dyes when the chromatin was colored by basic ones. The nucleolus has also been reported as staining much more lightly than the chromatin (YAMAHA and SINOTÔ 17). While these two constituents of the nucleus could thus readily be distinguished in the resting stage, it was generally impossible to separate them in dividing cells, for, as mitosis progressed, the staining properties of the chromatin became more oxyphilic (NAYLOR 11, KUWADA and SUGIMOTO 3). From the significant observation that the chromatin assumed certain staining characteristics of the nucleolus as the latter disappeared as

a discrete cell organ, it was inferred that the nucleolus contributed certain substances to the dividing chromatin.

There are certain disadvantages inherent in this technique of investigation. The specific staining reactions of both nucleolar material and chromatin were so conditioned by fixation as to be at times entirely reversed (ZIRKLE 19). Then, too, there was the more serious difficulty of separating these two substances, which are so intimately mixed in living cells, in fixed material where both were preserved. It was inevitable that each substance would obscure the other.

The present investigation is also carried out upon fixed material, and is of course subject to the limitations of all such work. The size, form, and chemical reactions of the nucleoli changed so greatly with variations in the fixatives, that it was not possible to ascertain their true structure by the methods here used. As no attempt was made to check the various fixation images with living sections, no conclusions could be reached concerning either the finer structure of the nucleoli or the exact amount and form of the nucleolar material. It was possible, however, by alterations in the fixing fluids, to fix: (1) the nucleolar material, chromatin, and mitochondria; (2) the nucleolar material and chromatin and dissolve the mitochondria; (3) the nuclear material and mitochondria and dissolve the chromatin; (4) the chromatin and dissolve or render incapable of staining the nuclear material and mitochondria; and (5) the nuclear material and dissolve the chromatin and mitochondria. The nucleolar material could thus be separated chemically from either the chromatin or the mitochondria, and its distribution in the cell investigated unobscured by either of the other two substances.

Whether the colorable material of the resting nucleus in the root tip of *Zea* is localized in the nucleolus or the nuclear reticulum, or in both, depends entirely upon the fixative used. As the substances in the two regions have such different chemical reactions to fixation, no useful purpose can be served by labeling them both chromatin, although both contribute material to the chromosomes. In the present paper the term chromatin will be limited to the stainable material of the reticulum, and is thus synonymous with basichromatin. This is the substance which forms the early spireme and determines certain of the staining properties of the chromosomes. The

nucleolus, by definition, contains no chromatin, and its contents will be referred to as nucleolar material or plastin. While there is very good presumptive evidence that the nucleolar matter is composed of two or more substances, as has been frequently maintained, the methods here used do not separate them readily, so they will be treated as forming a single unit.

The bibliography of the nucleolus is so extensive, and has been so well summarized, that not many references to the literature will be included in the present paper, nor will the present observations be correlated with the previous findings. The earlier work has been reviewed by MONTGOMERY (10), and later work by WALKER and TOZER (15) and LUDFORD (6); YAMAHA and SINOTÔ (17) and GUILLIERMOND and MANGENOT (2) have brought the references up to date. While the methods used in the present investigation may produce evidence of the nucleolar behavior not hitherto available, every specific finding has been both affirmed and denied by numerous workers.

The only tissue examined is the root tip of *Zea mays*, and the single stain used is Haidenhain's iron-alum haematoxylin. Definite conclusions concerning the composition or behavior of the nucleolus in general can hardly be drawn from so limited an investigation of its activities. The methods used, however, may be of more general interest.

#### Fixation of nucleoli

The effects of pH upon the fixation image of the bichromates has been recorded (ZIRKLE 18). When the fixing fluid is on the acid side of the critical range (pH 4.2-5.2) the image is essentially that of chromic acid; that is, the nucleolus, chromatin, spindle fibers, and spongioplasm fixed; nuclear lymph, mitochondria, and hyaloplasm dissolved. On the basic side of this range the nucleolus, nuclear lymph, mitochondria, and hyaloplasm are fixed and the chromatin and spindle fibers are destroyed. The change from one image to the other is as a rule sudden and complete, the two images being mutually exclusive and the exact point of change being determined by the specific cation. With the bichromates of copper (pH 4.6), glucinum (pH 4.6), and cerium (pH 4.8), the two images overlap with the fixation of chromatin, spindle fibers, nucleoli, nuclear lymph, mito-

chondria, and hyaloplasm. It will be noted that the nucleolus is fixed in both images; in the more acid with the chromatin, and in the one on the basic side of the range with the mitochondria. Nucleolar material can thus be separated from both the chromatin and the mitochondria.

In the acid fixation image the resting nucleus is a hollow body with a centrally located, darkly staining spherical nucleolus, and a periphery composed of the chromatin reticulum (fig. 26). The colorless halo surrounding the nucleolus is thus quite evident. In the more basic image the nucleus is a solid body composed of fixed nuclear lymph in intimate contact with a centrally located nucleolus (fig. 27). There is in this image no halo. The halo surrounding the nucleolus has been reported by numerous cytologists as a distinct structure in the living cell and by others as an artifact. Its existence in the root tips of *Zea* fixed with bichromates is determined entirely by the pH at which fixation occurs. BAILEY (1), however, using the same fixative throughout, has found that this halo exists about the nucleoli in the cambium of dicotyledons, but not about the nucleoli in the cambium of the gymnosperms, which suggests important differences between these two groups.

Unfortunately for an investigation of the nucleolar material unobscured by any chromatin in tissue fixed by the more basic bichromates, its form and distribution in dividing cells are greatly altered; in fact, no dividing cells are found in tissue thus fixed. If a dividing nucleus is so fixed that all chromosomes and spindle fibers are dissolved, the nuclear lymph rounded up and fixed as a single large droplet with the nucleolar material collected into one or two globules, the fixation image is that of a resting nucleus. As the mitochondria are also preserved by the fixatives, any nucleolar material in the cytoplasm cannot be identified with certainty.

The more acid bichromates in general fix the nucleolus in the resting nucleus as a single darkly staining sphere whose diameter is about half that of the nucleus. Mercuric bichromate is an exception, in that when it fixes the nucleoli they are stained brown by the fixative, and remain uncolored by the haematoxylin which stains the chromatin blue. It seems very significant that in material thus fixed and stained the later spireme has a light brown core and a dark blue periphery. When the root tips are fixed with copper bichromate



(pH 4.6) and stained with iron-alum haematoxylin, both chromatin and nucleolar material stain blue. If the sections are mordanted in the fixative, however, in place of the iron-alum the chromatin becomes blue but the nucleoli remain brown. Again the later spireme has a brown core (fig. 25). When fixed with ferrous, zirconium, uranyl, or mercurous bichromate the nucleoli lose their color in destaining before the chromatin. The central region of the nucleoli loses all color before the periphery. As a result they often appear to have a hull about them. This hull has frequently been described.

When the nucleoli are fixed with the more basic bichromates they are the darkest colored and most conspicuous objects in the section. They retain their stain longer than any other cell organ. Their shape and size are the same as when they are fixed by the more acid salts, except that when the alkali earths formed the cations of the fixatives the nucleoli were much smaller, and when lithium was the cation they were amoeboid in shape and had pointed pseudopodia (fig. 28).

Root tips fixed with the various acetates do not show the two distinct images which have been described for material fixed with the bichromates (19). However, changes in the pH at which acetate fixation occurs does cause certain very marked changes in the image. This change is primarily in the nucleolus. When the root tips of *Zea* are fixed with acetic acid, or any acetate more acid than pH 4.0, the nucleoli do not retain the haematoxylin stain and appear large, vacuolate, and colorless. Sometimes the vacuoles will remain faintly blue, although not so blue as the chromatin. The chromosomes are stained much darker than the resting chromatin; thus when chromosome counting is desired, it is possible to destain completely every other object in the section. This increase in the staining capacity of the chromatin as cell division progresses indicates that the difference in the staining properties of resting and dividing chromatin, noted particularly by NAYLOR (11) and by KUWADA and SUGIMOTO (3), is not due exclusively to the incorporation of nucleolar material in the latter, for with these fixatives the nucleolar material, at least in the nucleoli, is colorless. On the other hand, when the tissue is fixed with mercuric or copper bichromate, as has been stated, the dividing chromatin acquires certain of the specific staining reactions of the nucleoli.

On the basic side of pH 4.0 the acetates fix the nucleoli as much

smaller, solid, densely staining bodies. Chromatin is also fixed as long as the fixatives are more acid than pH 4.6. In more alkaline solutions the acetates can be divided into two groups, depending upon their fixing properties. Unlike the bichromates, neither group fixes mitochondria or nuclear lymph, no matter how alkaline the solution. When certain heavy metals form the cations, such as copper, mercury, uranium, and lead, chromatin is fixed by solutions more basic than any solution of bichromate which preserves chromatin. With certain other cations, silver, zinc, cadmium, nickel, etc., the root tip becomes a mere jumble with little recognizable except the nucleolus. Copper acetate (pH 5.2) often fixes resting chromatin in the form of globules. Such "chromatin nucleoli" are shown in fig. 1.

The best fixing fluids for nucleoli so far found are mixtures of bichromates and acetates. The acetates seem to penetrate the tissue more rapidly and thus primarily determine the fixation image. The bichromates harden the image and enable it to pass through the processes of dehydration, imbedding, and sectioning relatively unaltered. The more basic acetates alone, with the exception of those formed by certain of the heavier metals, do not harden the tissue sufficiently to preserve it through the subsequent treatment. The mixtures of acetates and bichromates have the great advantage as nucleolar fixatives of dissolving all mitochondria; thus in the material they fix there is no danger of confusing any fragment of nucleolar material extruded in the cytoplasm with the mitochondria.

An investigation of chromate and acetate fixation brings to light the importance of the cation in the preservation of the nucleolus. Most of the fluids designed to fix chromatin have no cation except hydrogen. These solutions are satisfactory preservatives of chromatin, but are quite erratic in the fixation of nucleoli. When the cation of a chrome-acetic mixture is one of the group of heavy metals previously mentioned, both chromatin and nucleoli are well fixed and mordanted. The connection between these two substances can then easily be seen. Copper-chrome-acetate (pH 4.6) fixes the chromatin excellently, and so hardens the nucleoli that they cannot be cut, but are thrown out of the cell when hit squarely with the microtome knife. With certain other elements forming the cations of

the relatively basic chrome-acetate mixtures, all chromatin is dissolved and only the nucleolar matter is well preserved and mordanted. There is none of the clumping of nucleolar material such as occurs when fixation is effected by the more basic bichromates. Thus in root tips fixed with nickel-chrome-acetate (pH 5.0-5.2), the nucleolar material can be followed through all of the mitotic phases unobscured by either chromatin or mitochondria.

The addition of formaldehyde greatly influences the fixation images of the acetate. A 2 per cent solution of acetic acid (pH 3.6) fixes the nucleoli as large, vacuolate bodies which do not retain the haematoxylin stain. A mixture of 4 per cent formalin and 2 per cent acetic acid (pH 3.6) fixes the nucleoli as a solid, densely staining body (fig. 2). The image given by a 2 per cent solution of sulphuric acid is not thus altered by the addition of formalin, as the nucleolus thus fixed remains unstained (fig. 3). The mixture of sulphuric acid and formalin fixes in some respects like mercuric and copper bichromate, in that the later spireme and the chromosomes show a core as colorless as the nucleolus in resting cells.

The fixing fluids used in the present investigation are as follows:

1.  $\text{Cu}(\text{C}_2\text{H}_3\text{O}_2)_2$ ..... 5 gm.  
 $\text{H}_2\text{O}$ ..... 100 cc., pH 5.2 (fig. 1).
2.  $\text{HC}_2\text{H}_3\text{O}_2$ ..... 2 cc.  
 Formaldehyde 40 per cent.. 10 cc.  
 $\text{H}_2\text{O}$ ..... 90 cc., pH 3.6 (fig. 2).
3.  $\text{H}_2\text{SO}_4$ ..... 2 cc.  
 Formaldehyde 40 per cent.. 10 cc.  
 $\text{H}_2\text{O}$ ..... 90 cc., pH < 1.0 (fig. 3).
4.  $\text{HC}_2\text{H}_3\text{O}_2$ ..... 2.5 cc.  
 $\text{CrO}_3$ ..... 2.5 gm.  
 $\text{CuO}$ ..... Slight excess  
 $\text{H}_2\text{O}$ ..... 100 cc., pH 4.6 (figs. 4, 6-13).
5.  $\text{HC}_2\text{H}_3\text{O}_2$ ..... 2 cc.  
 $\text{HgO}$ ..... Slight excess  
 $\text{H}_2\text{O}$ ..... 100 cc., pH 4.2 (figs. 5, 14).
6.  $\text{U}(\text{C}_2\text{H}_3\text{O}_2)_4$ ..... 5 gm.  
 $\text{H}_2\text{O}$ ..... 100 cc., pH 5.0 (fig. 17).

- |     |   |  |
|-----|---|--|
| 7.  | $\text{HC}_2\text{H}_3\text{O}_2$ ..... | 2.5 cc.                                |
|     | $\text{CrO}_3$ .....                    | 2.5 cc.                                |
|     | $\text{Ni}(\text{OH})_2$ .....          | Slight excess                          |
|     | $\text{H}_2\text{O}$ .....              | 100 cc., pH 5.0 (figs. 15, 16, 18-24). |
| 8.  | $\text{CrO}_3$ .....                    | 2.5 gm.                                |
|     | $\text{UO}_2$ .....                     | Slight excess                          |
|     | $\text{H}_2\text{O}$ .....              | 100 cc., pH < 1.0 (fig. 26).           |
| 9.  | $\text{Cr}_2\text{O}_3$ .....           | 2.5 gm.                                |
|     | $\text{SrO}$ .....                      | Slight excess                          |
|     | $\text{H}_2\text{O}$ .....              | 100 cc., pH 5.8 (fig. 27).             |
| 10. | $\text{Cr}_2\text{O}_3$ .....           | 2.5 gm.                                |
|     | $\text{Li}_2\text{CO}_3$ .....          | 3.0 gm.                                |
|     | $\text{H}_2\text{O}$ .....              | 100 cc., pH 5.2 (fig. 28).             |
| 11. | $\text{CrO}_3$ .....                    | 2.5 gm.                                |
|     | $\text{CuO}$ .....                      | Slight excess                          |
|     | $\text{H}_2\text{O}$ .....              | 100 cc., pH 4.6 (fig. 25).             |

#### Behavior of nucleolar material during cell division

The plastin in the resting nucleus is concentrated in a single globule, the nucleolus. In root tips fixed with the more basic bichromates, the apparently resting nucleus often contains two or three nucleoli; but evidence has already been cited which indicates that these nuclei are the remains of dividing ones whose chromosomes and spindle fibers have been dissolved, and whose nuclear lymph and plastin have been agglutinated. Whether any strands or fibers connect the nucleolus with the chromatin reticulum in the living resting nucleus cannot be stated. Certainly none exists in those fixed with the more acid bichromates, where the nucleolus is surrounded by a clear, colorless halo, or in those fixed with the more basic ones, where the nucleolus is in intimate contact with the fixed nuclear lymph and the chromatin reticulum is dissolved. No connection exists in acetate-fixed nuclei. When lithium bichromate is used as a fixative, distinct strands connect the tips of the "pseudopods" of the amoeba-shaped nucleolus with the nuclear membrane, but this image departs greatly from the form in the living cell.

In the early prophase, however, a distinct connection is estab-

lished between the nucleolus and the spireme (fig. 2). The connection rapidly broadens (figs. 3, 4), and the nucleolus becomes pear-shaped, with its smaller end connected to the spireme (figs. 6, 7). WAGER (14) has shown this connection quite clearly. By far the best fixatives for the study of this stage are those which preserve both chromatin and plastin, those giving the best results being copper-chrome-acetate (figs. 4, 6-13) and mercuric acetate (figs. 5, 14). Often the smaller end of the nucleolus splits, and thus a double connection with the spireme is established (figs. 5, 8, 15). There is every appearance of the nucleolar material flowing into the spireme, as has been reported from other material by LENOIR (4) and MARTENS (8).

There is also chemical evidence of the nucleolar material entering into the spireme. When the cell is fixed with mercuric bichromate the nucleoli are stained brown by the fixative, and are not colored by Haidenhain's haematoxylin. Later spiremes so fixed and stained have a brown core. Likewise, when the cell is fixed with copper bichromate and mordanted with the fixative in place of iron alum, the nucleoli and the core of the spireme are brown, while the chromatin reticulum and the periphery of the spireme are stained blue. When the cell is fixed with a mixture of formalin and sulphuric acid, the chromatin reticulum and the periphery of the spireme and chromosomes are stained blue with haematoxylin; but the nucleoli and core of the spireme and chromosomes remain colorless. This evidence indicates not only that the nucleolar material enters into the spireme, but that it does so in a particular way, flowing into the spireme just as water does into a collapsed rubber tube. Unfortunately for the hypothesis of this distinct special relationship between the nucleolar material and the chromatin, it is directly contradicted by evidence derived from other fixation. When the root tips are fixed with zinc or nickel bichromate, the nucleoli of resting nuclei are the heaviest staining objects in the cells. Chromosomes thus fixed have colorless cores.

As cell division progresses the chromatin acquires the staining properties of the nucleolus. This has been very clearly shown by NAYLOR (11) and by KUWADA and SUGIMOTO (3). The latter workers ascribe this change in the chromatin's staining capacity to the disappearance of the nuclear membrane. It could equally well be

explained by its taking up of nucleolar material. This latter hypothesis is not necessarily the explanation, however, for when the tissue is fixed with any acetate on the acid side of pH 4.0, the nucleolar material, at least in the resting cells, loses the haematoxylin stain before the chromatin, yet the dividing chromatin retains the stain longer than the resting. The crucial evidence for the nucleolar material entering into the spireme is to be found in cells fixed with nickel-chrome-acetate (pH 5.0-5.2). This fixative dissolves all chromatin and mitochondria, and preserves the nucleolar material. The plastin can thus be seen entering into the spireme unobscured by any chromatin (fig. 22).

The nucleolus, then, connected at two places with the spireme, becomes drawn out into a rod (fig. 9). This rod lies regularly at right angles to the equatorial plate. It becomes drawn out (figs. 10, 16, 17) and dumbbell-shaped (figs. 11, 18). As YAMAHA and SINOTÔ (17) have reported, it then constricts in two. The two parts round up and pass to the poles of the spindle (fig. 12), often remaining connected to the equatorial plate by a darkly staining thread (figs. 13, 19). The thread then disappears and the division is complete (fig. 14). Fortunately for chromosome counting, these bits of plastin do not linger at the equatorial plate.

From the preceding discussion it is evident that the nucleolar material enters the daughter cells in two distinct ways. It is carried over in the chromosomes and also passes over in a distinct body, the latter form reaching the poles first. YAMAHA and SINOTÔ state that this globule of plastin at the pole fragments and all of the pieces pass out into the cytoplasm. The expulsion of nucleolar fragments into the cytoplasm has also been reported recently by LENOIR (5). In *Zea* the globules of plastin at the poles of the spindle can be observed to fragment, and the fragments can be observed in the cytoplasm, although whether they all go out of the nucleus or not cannot be stated; the majority certainly seem to. There is no danger of confusing these nucleolar granules in the cytoplasm with the mitochondria when the material is fixed with nickel-chrome-acetate, as this fixative dissolves all of the latter. Some of these granules are shown in fig. 14.

These nucleolar granules pass into the cytoplasm while the

chromosomes are at metaphase. At this stage the nucleolar material is very thoroughly scattered throughout the cell, that is, in the granules and in the several chromosomes. Figs. 23 and 24 show cells whose chromatin and mitochondria have been dissolved. They are shown in cross-section. The masses of darkly staining granules in the center are of nucleolar material in the chromosomes at the equatorial plate. The granules in the cytoplasm are of the same substance.

As the chromosomes separate at anaphase and go to their respective poles they carry their contained nucleolar material with them (upper cell in fig. 19); thus the second lot of plastin arrives at the poles. As the chromosomes unite to form the daughter nuclei, and as the nuclear membranes are formed, the nucleolar material collects into several droplets (figs. 20, 21). These droplets, united by stainable threads, flow together and form the nucleolus of the resting cell.

The plastin thus is continuous from cell generation to cell generation, like the chromosomes, and like them is formed by an increase in the amount of pre-existing material. In every cell division, however, a certain amount of it, perhaps merely an excess amount, fragments and passes out into the cytoplasm in the form of granules which ultimately disappear.

### Discussion

The question naturally arises as to what rôle the nucleolar matter plays in the economy of the cell. The numerous answers quoted in the beginning of this paper evince the interest it has held for cytologists. It is perhaps impossible to determine this function by a mere observation of the behavior of the plastin during cell division, and it seems probable that its discovery will have to await the development of new lines of attack. The fortunate circumstances which allowed the chromosomes to be investigated by both the genetic and cytological techniques have increased our knowledge of these bodies out of all proportion to our knowledge of the other nuclear constituents.

Considering how widespread the distribution of nucleoli is throughout the animal and plant kingdoms, they would normally

be expected to perform some universal function. This would not prevent them, of course, from performing in addition certain secondary functions in relatively specialized tissue, such as giving rise to yolk granules in egg cells. We should, however, look for some activity common to all nucleoli to find their primary function. Considering how little is really known at present about the nucleolar material, it would seem that any further speculation concerning its rôle in the cell would be futile, and would be useful only if it served to keep the question open.

During cell division some nucleolar material enters into the chromosomes and is presumably in intimate contact with the genes. In the next division a part of this material passes out into the cytoplasm and disappears. It is tempting to see in this a mechanism for carrying the influence of genes to the organism. There is a specific connection between the nucleoli and the chromosomes, as has been shown by DEMOL (9), who found regularly in *Hyacinthus orientalis* two nucleoli in diploid, three in triploid, and four in tetraploid forms. It must be remembered, however, that there is no evidence as yet that the genes need any such mechanism.

The platin may serve merely as a framework for the distribution of chromatin to the daughter cells. In resting nuclei the chromatin is electro-negative and the nucleoli are electro-positive (KUWADA and SUGIMOTO 3, WILSON 16). The poles of the spindle form and the electro-negative spireme retreats to the equatorial plane, the maximum distance from the two poles. There it is permeated by the electro-positive nucleolar material until it is mostly electro-positive; whereupon it fragments into chromosomes which split. The chromatin, now bearing a different charge, reverses its previous motion and migrates to the two poles. That the electro-positive nucleolar material is attracted to the poles is shown by the fact that that portion of it which does not enter the spireme is pulled apart into two masses which migrate exactly to the poles (figs. 12-14).

This does not explain why the globules of nucleolar material, once they have reached the poles, should fragment and pass out into the electro-positive cytoplasm; nor does it account for any function they might perform there. It also presupposes that the spireme flattens out on the equatorial plane before it breaks up into chromo-



somes. More definite knowledge concerning this possible function of the nucleolar material should be obtained by further study of the forces involved in cell division.

### Summary

By the use of different fixatives it was found possible to fix: (1) chromatin, plastin, and mitochondria; (2) chromatin, plastin, and dissolve all mitochondria; (3) plastin and mitochondria, and dissolve the chromatin; (4) chromatin, and dissolve or render unstainable all plastin and mitochondria; and (5) plastin, and dissolve all chromatin and mitochondria. It was thus possible to investigate the behavior of the nucleolar material during cell division unobscured by any chromatin or mitochondria. In the resting nucleus all chromatin is localized in the reticulum, the nucleolus containing none. After the spireme is formed the nucleolus becomes connected with it, and later becomes pear-shaped, the point of attachment being at the smaller lobe of the pear. As the spireme flattens out on the equatorial plane nucleolar material flows into it. The smaller lobe of the nucleolus is drawn out and splits in two, giving the nucleolus two connections with the spireme. As the nucleolus loses material it becomes rod-shaped, and finally lies at right angles to the plane of division. It is drawn out, constricted in two, and the two fragments round up and migrate to the poles. There they break up into small granules, some of which, possibly all, pass into the cytoplasm where they later disappear. In telophase the nucleolar material which had been incorporated in the chromosomes collects into small globules which later fuse to form the daughter nucleolus. Nucleolar material is thus continuous, and is derived from previously existing nucleolar material. Some, however, is apparently lost during each cell division. Two possible explanations of the function of the nucleolar material are added to those already proposed. (1) The plastin, by entering into the chromosomes, coming into intimate contact with the genes during cell division, and passing in part out into the cytoplasm during the subsequent division, may serve as a vehicle for transmitting the influence of the genes to the organism. (2) The plastin, being electro-positive, changes the electro-negative spireme by flowing into it, to an electro-positive chromatin complex;

thus the chromatin, which had collected at the equatorial plate as far as possible from the poles of the spindle, reverses its motion with its electrical charge and migrates to the two poles.

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### EXPLANATION OF PLATES XII, XIII

All figures are taken from longitudinal sections of root tips of *Zea mays* except figs. 22, 23, and 24, which are from cross-sections. Haidenhain's iron-alum haematoxylin is the only stain used. Magnification,  $\times 1200$ ; no photographs retouched.

#### PLATE XII

FIG. 1.—Fixed with  $\text{Cu}(\text{H}_2\text{C}_3\text{O}_2)_2$  pH 5.2, showing resting chromatin changed into "chromatin nucleoli."

FIG. 2.—Fixed with mixture of acetic acid and formalin, showing connection between nucleolus and spireme.

FIG. 3.—Fixed with sulphuric acid and formalin, showing connection between pear-shaped lightly staining nucleolus and darkly staining spireme.

FIG. 4.—Fixed with copper-chrome-acetate pH 4.6, showing connection between nucleolus and spireme.

FIG. 5.—Fixed with mercuric acetate pH 4.2, showing split in small end of pear-shaped nucleolus and double connection between it and spireme.

FIGS. 8-10.—Fixed with copper-chrome-acetate, showing rod-shaped nucleolus moving to position at right angles to equatorial plate.

FIGS. 11-13.—Fixed with copper-chrome-acetate, showing division of nucleolar material; globules arrive at poles while chromatin is still at metaphase.

FIG. 14.—Fixed with mercuric acetate, showing chromatin at metaphase and globule of plastin at pole; some fragments of nucleolar material in cytoplasm.

#### PLATE XIII

FIG. 15.—Fixed with nickel-chrome-acetate pH 5.2, which preserves plastin but dissolves all chromatin; nucleolus shown attached to spireme.

FIG. 16.—Fixed with nickel-chrome-acetate, showing nucleolar material at right angles to spireme.

FIG. 17.—Fixed with uranium acetate pH 5.0, showing nucleolus attached to spireme.

FIG. 18.—Fixed with nickel-chrome-acetate, showing nucleolar material divided but still at right angles to equatorial plate.

FIG. 19.—Fixed with nickel-chrome-acetate, showing (1) two masses of plastin in anaphase in upper cell; and (2) globules of nucleolar material passing to poles prior to division of chromosomes.

FIGS. 20, 21.—Fixed with nickel-chrome-acetate, showing plastin globules in daughter nuclei before fusing to form daughter nucleoli.

FIG. 22.—Cross-section fixed with nickel-chrome-acetate, showing nucleolar material passing out into spireme.

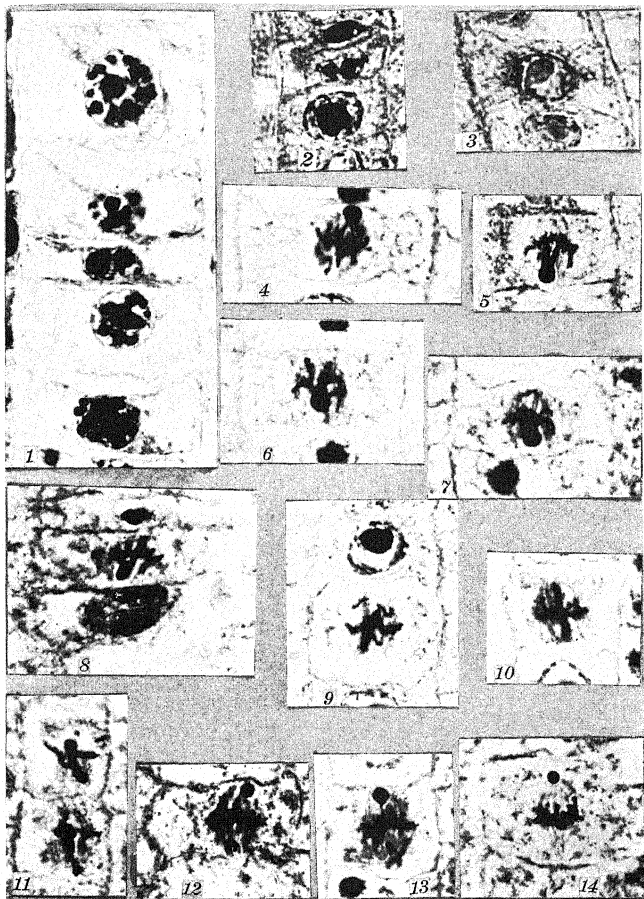
FIGS. 23, 24.—Cross-section fixed with nickel-chrome-acetate, showing polar views of equatorial plate; dark masses in center of cells composed of nucleolar matter left behind by dissolved chromatin; cytoplasmic granules also of nucleolar matter.

FIG. 25.—Fixed with copper bichromate pH 4.6 and mordanted with fixative instead of iron-alum; lighter regions of spireme of nucleolar material.

FIG. 26.—Fixed with uranyl bichromate pH < 1.0, showing halo about nucleoli.

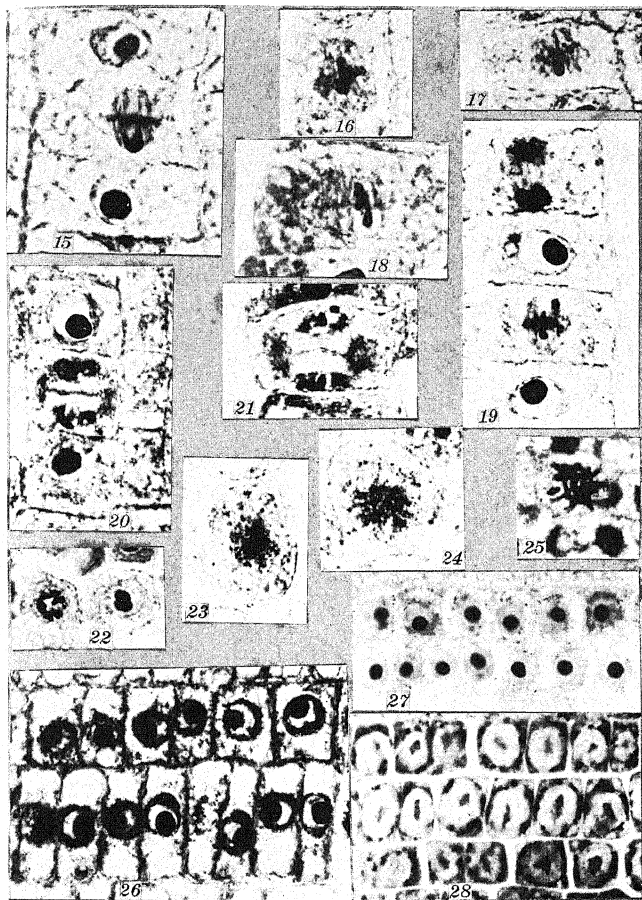
FIG. 27.—Fixed with strontium bichromate pH 5.8, showing absence of halo and chromatin.

FIG. 28.—Fixed with lithium bichromate pH 5.2, showing amoeba-shaped nucleoli.



ZIRKLE on ZEA MAYS





ZIRKLE on ZEA MAYS





## PELVETIA FASTIGIATA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 387

LAURA BROOKS MOORE

(WITH TWENTY-FIVE FIGURES)

### Introduction

In a study of a western American species of *Pelvetia*, the writer observed that the conceptacles occurred, not only at the fruiting tips of the shoot, as in *Fucus*, but also much farther down, being rather numerous as far as the second and third forks, and scattered sparsely still farther from the growing tips. These conceptacles were found to contain well developed oogonia. As this condition does not occur in the European species, *Pelvetia canaliculata*, this investigation was undertaken for the purpose of determining the nature of the scattered conceptacles and their relation to hair pits. Other problems arose, resulting in the study of the apical cell, the anatomy of the thallus, and the development of the conceptacle and the sex organs of *Pelvetia*.

The genus *Pelvetia* was founded by DECAISNE and THURET in 1845 (3). J. G. AGARDH assigned to *Pelvetia fastigiata* its specific name in 1848. During the following 25 years no work on *Pelvetia* was published. In 1875, KNY (7) described the apical cell of *P. canaliculata*, and in 1878 THURET (17) published his classical work on the conceptacle of *Fucus serratus* (L.), *F. vesiculosus* L., and *F. platycarpus* Thuret; *Ascophyllum nodosum* (L.) Le Jolis, *Pelvetia canaliculata* (L.) Lyngb., *Bifurcaria tuberculata*, and *Cystoseira fibrosa* (Huds.) Ag. All species of *Fucus* were shown to develop 8 oospheres in each oogonium; *Ascophyllum* was shown to develop 4 eggs; *Pelvetia*, 2; and each of the other genera one oosphere in each oogonium.

OLTMANN (12) was the first to investigate the Fucaceae adequately from a morphological standpoint. His work was revised in 1904, and further revised in 1922-1923. He confirmed the work of THURET but extended his investigations much further, finding that

8 nuclei are formed in the oogonium of *Pelvetia canaliculata*, and that 2 become centers for oospheres, while 6 are extruded at the equator. OLTMANNS studied the development of the conceptacle carefully in many species of the Fucaceae, but not in *Pelvetia*. He also made thorough investigations of the development of the sporplings of many Fucaceae, including that of *P. canaliculata*, which he found to be like the other Fucaceae in general. There is a 3-sided apical cell which becomes 4-sided with the broadening of the thallus, but from this point the development is unlike that of *Fucus*. *Fucus* stands nearer the common ancestor because its thallus is comparatively simple; its higher number of eggs points to an earlier origin. *Pelvetia* is more highly developed in that the number of eggs has been reduced to two, but in anatomical structure it has not gone so far as *Fucus*. This is another case, according to OLTMANNS (13), where sex organs have progressed and vegetative organs have stood still or retrograded.

The development of the conceptacle in the Fucaceae has also been studied successively by REINKE, who worked on species of *Fucus*; by BARTON (1), who studied *Turbinaria*; and by Miss ROE (14), who investigated the conceptacle in species of *Fucus* and in *Splachnidium*. The most important contribution was made by BOWER (2), however, working on various species of the Fucaceae, including *Pelvetia canaliculata*, work which has been generally accepted. The earlier investigators held with KÜTZING that the conceptacle was derived from the epidermal layer of the thallus, but the others confirm for the most part the views of BOWER, who found that the conceptacle was the product of basal cells cut off from the epidermal cells, together with cortical cells. Miss SIMONS (15) differs radically from all of them in her claim that a single initial of the epidermal layer gives rise to the entire inner wall of the conceptacle from which sex organs spring.

The first cytological work was that of FARMER and WILLIAMS (4). They studied chiefly species of *Fucus* and *Pelvetia canaliculata* in a supplementary way, giving special attention to egg formation in the oogonium, the third division, fertilization, and early segmentation divisions.

STRASBURGER (16) made a cytological study of the third division

in the oogonium of *Fucus platycarpus*, and fertilization in *F. serratus* and *F. vesiculosus*. Most of our knowledge of the cytology of these algae has as its basis the brilliant work of these men.

YAMANOUCHI (18) published a paper on mitosis in *Fucus* based upon *F. vesiculosus*, and sums up the results of his study as follows: The chromosome number is 64, reduced at the end of the second nuclear division in the oogonium and antheridium initials; each of the 4 nuclei at the end of the first 2 divisions contains 2 univalent chromosomes, and this number persists until the formation of the sperm and egg; the phase containing 32 chromosomes may be regarded as the gametophyte generation, and the 64-chromosome phase after the fusion of the gametes as the sporophyte generation.

The American species, *Pelvetia canaliculata*, was studied by HOLTZ (6). He investigated the development of the conceptacle, the apical cell, and the formation of the oospheres. His views on the development of the conceptacle differ from those of BOWER, in that HOLTZ thought that the basal segments of the epidermal cells and also 5 or 6 rows of the cortex beneath them disintegrate, as well as a row of epidermal cells; and that the conceptacle cavity is formed mainly from cortical cells. HOLTZ represented the division of the contents of the oogonium in the same plane as the European species, *Pelvetia canaliculata*. He was able to see only 4 nuclei in each egg.

NIENBURG (10), in an article on the development of the conceptacle in the Fucaceae, criticized HOLTZ' conclusions as to the manner in which the conceptacle of *Pelvetia fastigiata* is developed. NIENBURG secured the American species from California and made a careful study of it. He concluded that the conceptacle arises from a single surface cell which remains behind its neighbors in cell division, so that very early it is sunken. The initial does not have a flask shape, as in other Fucaceae, but is rather even in breadth and ends in a rounded point. Also, in the 2-nucleate stage the nuclei do not lie side by side. The first division is by a longitudinal wall; then follows, first in one and then in the other initial half, a transverse division. From the two basal cells arises through irregular cell divisions the floor of the conceptacle. The upper portion of the initials grows out into short hairs. The side walls begin to be formed through division of the neighbor cells, as in *Fucus*. That other cells

assist in forming the older stages is not certain but probable, according to NIENBURG. He agrees with SIMONS (15) that there is no dying off nor disintegration of cells; but SIMONS thought that the cortex played no rôle in the formation of the conceptacle wall, while NIENBURG claimed that the side walls of the conceptacle originated from the cortex and basal cells of the epidermis. NIENBURG does not mention the peculiar "tongue" cell and "cup-shaped" cell which SIMONS found in *Sargassum*.

KYLIN (8) contributed two interesting articles on the structure of sperms in the Fucaceae. The nucleus divides until 64 are formed, as shown by YAMANOUCI (18). In the 2- and 4-nucleate, and also in the 64-nucleate stages he observed orange chromatophores, one for each nucleus. In the chromatophores of the Fucaceae, according to KYLIN, there are 3 dyes besides chlorophyll: carotin, xanthophyl, and fucoxanthin, only the first two being found in the eyespot of the sperm. Sperms of *Pelvetia* have no eyespots, however, according to OLTMANN.

#### Investigation

The western American species was identified by the writer as *Pelvetia fastigiata* (J. Ag.) de Toni. This genus was founded upon the *Fucus canaliculata* of LINNAEUS (Syst. Nat. II. 1759. p. 716), based largely upon the fact that the oogonium produces but 2 viable eggs instead of 8, the characteristic number for *Fucus*. Eight nuclei are formed within the oogonium, but 6 of them are extruded. The eggs are not fertilized outside of the oogonium as in *Fucus*. The fronds are firm and flattened, becoming terete with age toward the base, and arise from a disk-shaped holdfast; there is no midrib; the branching is dichotomous, diffuse, and fastigiate; the growing point is apical; reproductive organs are developed in conceptacles; oogonia develop two eggs; it is monoecious; color is yellowish brown to dark olive-green.

*Pelvetia fastigiata* is always found growing on rocks a little below the tide line, where it is sprayed by the surf. It is submerged not more than three hours out of twelve, and OLTMANN (12) suggests that it might be classed as a land plant for this reason. Its range extends from Coos Bay, Oregon, to the west coast of lower California. It is probably the same species as the *P. wrightii* of Japan.

Plants of *Pelvetia fastigiata* vary in size. At San Pedro, plants 10 dm. long have been observed. On the west coast of the Monterey Peninsula, the type locality, the branches are wide-spreading, angles rounded, and some of the fronds measure 2 cm. wide near the base.

The material for this study was collected by Professor C. J. CHAMBERLAIN near Redondo, California, in September, 1927; and by Dr. A. W. HAUPT at three different locations at different times, namely, San Pedro (October, 1924), Paleo Verdeo (February, 1926), and at Laguna (July, 1926). The material was stained with Haidenhain's iron-alum haematoxylin.

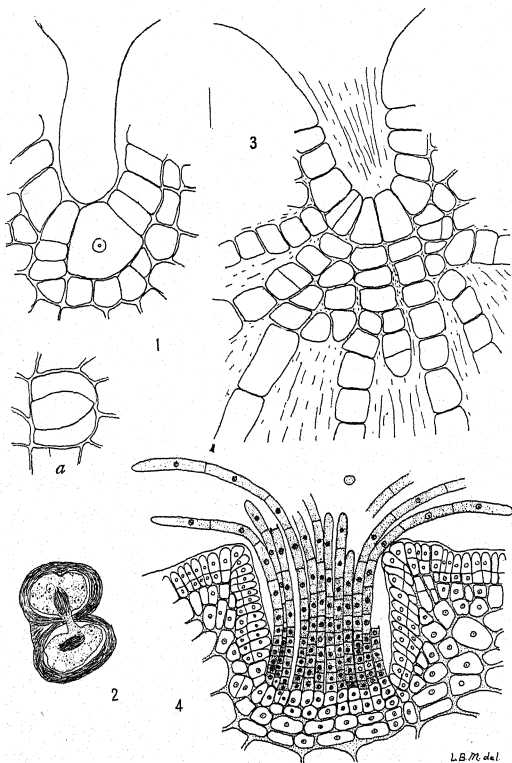
#### ANATOMY OF THALLUS

Only adult material was used. Attention was given to the apical cell, the general structure of the thallus, hair pits, and conceptacles.

**APICAL CELL.**—The form of the apical cell is a four-sided, truncated pyramid. The first segment cut off is basal; then follow walls to the right and left which cut off two segments from the opposite sides of the pyramid. Next a wall comes through to one side of the center at right angles to two and three. The segments thus formed are usually wider than the preceding lateral segments, which makes the apical cell longer than wide as seen in longitudinal sections (fig. 1). The segments are not angular but have convex walls, probably due to the greater density of their contents (fig. 1 a). The apical cell is sunken in a cleft at the apex, with sides parallel to the flattened surface of the thallus, as in other Fucaceae. This cleft is not so deep in *Pelvetia*, however, and its bottom has a more broadly rounded contour. The cleft and the mucilage which fills it give protection to the tender growing point. The depression is formed by growth as the segments are cut off from the apical cell on all four sides.

**THALLUS.**—There are three regions of the thallus: the epidermal region, the cortex, and the central cylinder or pith region.

The epidermal layer consists of a single row of palisade cells, much longer than wide, and occurring in pairs, due to their mode of longitudinal division. An epidermal cell first cuts off a nearly cubical basal portion; then the upper portion divides longitudinally, resulting in extremely long slender cells, while the basal cell usually does not divide in this plane, so that we have twin epidermal cells



FIGS. 1-4.—Fig. 1, apical cell in longitudinal section, *a*, in transverse section ( $\times 415$ ); fig. 2, cells of cortex ( $\times 635$ ); fig. 3, apical region showing pith filaments ( $\times 415$ ); fig. 4, hair pit ( $\times 415$ ).\*

\*All drawings reduced one-half except figs. 19-25 which are reduced one-third.

with one basal cell. The basal cell then cuts off segments from its lower surface. The palisade cells are rich in plastids and carry on photosynthesis; also, some plastids occur in the basal cells and even in the cortex. The plastids are oval or ellipsoid, regular in size, and are packed very closely in the epidermal cells. The epidermis is covered by a thick sheath of mucilage.

The cortex has its origin from the basal segments of the epidermal cells. The cells are quite irregular in size but always larger than the basal cells. They are rounded, and the intercellular spaces are filled with mucilage. Their function is storage and conduction (fig. 2).

The central region is a very loose web of filaments of large, elongated pith cells. *Pelvetia* has no midrib, and the central cylinder is composed of mucilage and pith filaments in about equal proportion, the mucilage showing distinct striations in stained preparations. There are infrequent air spaces but no air vesicles, and, as *Pelvetia* is not in water for more than a fourth of the time, air vesicles are not especially needed. The filaments of pith cells are from two to six cells long, sometimes longer. The cells are rectangular, and have very thick but delicate walls formed by successive thin layers or lamellae, like walls of laminated bast fibers (fig. 3). They are not thickened in the way that conductive cells are thickened, but a series of delicate layers seems to have been laid down by the cytoplasm. There is evident protoplasmic connection between the cells of the web through large openings in the cell walls, which are easily seen, and through which the protoplasm passes. This also occurs in the cortical cells. All of the pith cells have nuclei. The function of the pith filaments seems to be conduction, and in lower parts of the stem they have the pointed ends characteristic of conductive cells. The filaments are branched, and form an irregular network traversing the mucilage which fills the central region, some filaments ending blindly in the mucilage. Pith filaments arise from the basal segment of the apical cell.

Hypphae are confined to the holdfast and a space 2 or 3 cm. above it in the stele. They are the strengthening fibers that give mechanical support; and in many Fucaceae that grow where they are swept by violent waves hypphae continue the length of the plant.

*Pelvetia* is not subjected to this strain and is not provided with the strengthening tissue. Its vegetative tissue is simple, with little differentiation.

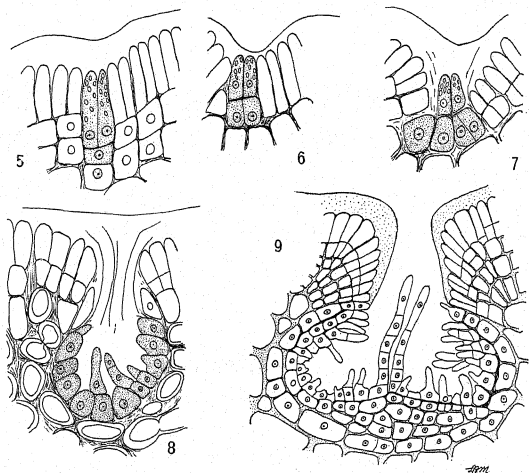
**HAIR PITS.**—*Pelvetia fastigiata* shows a few indubitable hair pits, although they have never been reported for this genus (fig. 4). They differ from fertile conceptacles in several particulars and may readily be distinguished. They are of different shape. A median cross-section through a conceptacle shows a bowl-like form with a comparatively narrow mouth, without which the conceptacle would be spherical. The hair pit has a broad mouth and straight side walls in transverse section, and the bottom is almost straight across, so that it is rather rectangular than spherical. No conceptacles of this shape were found. Again, the hairs of the hair pit are larger in diameter and are full of darkly staining contents, while the hairs found in conceptacles are smaller and nearly colorless. The longer hairs of the conceptacles of *Pelvetia* grow more commonly on the sides near the mouth of the conceptacle; whereas in the hair pit the hairs have their vegetative bases in the floor of the pit. The hairs of pits project several times their own length, while the hairs of conceptacles rarely extend much, if any, beyond the ostiole. The hairs of hair pits are divided into cells, short at the base, and gradually longer toward the apex. The base broadens into a vegetative basal cell imbedded in the floor of the pit. Their function probably is to increase the absorptive capacity of the plant.

**CONCEPTACLES.**—Conceptacles are located at the tips of fronds, as in *Fucus*, but the fruiting portions do not present so much of a swollen appearance, and there are also many conceptacles as far down as the second and third dichotomy, and a few scattered ones below.

The initial cell appears depressed, on account of more rapid growth of the neighboring cells, and there arises a slight depression in the mucilage layer surrounding the epidermis. Next, the initial cell divides longitudinally (fig. 5); both daughter cells cut off basal cells; the basal cells continue to divide and form the wall of the bottom of the conceptacle (fig. 6); meanwhile division and growth take place in the neighboring cells. The pair of initial cells is soon at the bottom of a pit twice their length and growing deeper, the



sides of which are formed at first by epidermal cells forced to assume new positions by the great activity of growth taking place in the bottom of the forming conceptacle (fig. 7). These epidermal cells form the walls of the upper part of the conceptacle which eventually



FIGS. 5-9.—Fig. 5, early stage of conceptacle formation; fig. 6, second stage showing division of basal cell; fig. 7, each half of basal cell divided; fig. 8, later stage, shaded cells of floor of conceptacle are derivatives of basal cell; fig. 9, later stage: ostiole lined with epidermal cells, floor of conceptacle and genetic layer are from basal cell, upper cells of the two initials divided to form hairs, sex organ initials and hairs beginning to appear ( $\times 415$ ).

is the ostiole, and the basal segments of the initials form the entire inner wall from which later the sex organs come (fig. 8). The shape of the young conceptacle in early stages is similar to the shape of the groove at the apical region when seen in cross-section, which is not surprising because the same sort of activity is present there. In maturity the conceptacle becomes globular except the ostiole or

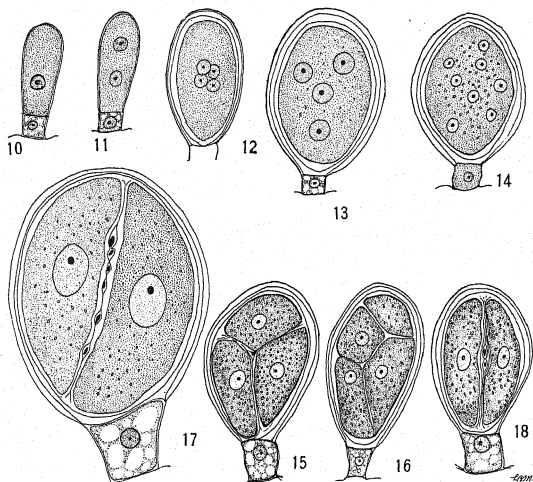
opening, which is narrow. The divisions of the basal segments of the initials form a mound in the bottom, and there are various protruding initials in the early stages (fig. 9); but at the stage of maturity when oogonia and antheridia are ripe the floor is even and its cells are flattened. The upper segments of the initials also divide and grow into two filaments several cells in length, which function apparently as hairs in the conceptacle (fig. 9). They pass through the life cycle of normal hairs, gradually losing their contents, then their transverse walls, and finally becoming colorless sheaths. This is the history of other hairs of the conceptacle. The only differences noted were in size, the hairs from the initials being larger than any other hairs in the bottom of the conceptacle, and an occasional tendency to divide laterally, thus forming a complex of cells.

There are three kinds of hairs found besides those arising from the initials: the long colorless hairs near the ostiole, the paraphyses on which antheridia are sometimes borne, and short hairs. The last are seen also in hair pits, and are found in the young stage of conceptacles but not in the mature stage. Paraphyses are not numerous and will be discussed later in connection with antheridia. None of the hairs are present in great numbers; in fact, *Pelvetia* is not a hairy plant, and this makes it the more surprising that hair pits are found.

The conceptacles located lower down on the fronds are full of cells, and it is concluded that they started as conceptacles but developed vegetatively, although they may have functioned as conceptacles and then filled up. Of course they are older in the life history, because conceptacles originate only at a growing point.

SEX ORGANS.—Oogonium initials appear early in the development of the conceptacle, and can be recognized by their greater breadth as compared with other initials. They divide into two cells, the lower of which becomes the stalk cell; the upper develops into the oogonium (fig. 10), containing at first a single nucleus which divides while the oogonium cell is still slender, but has elongated considerably (fig. 11). The oogonium then swells and becomes oval and the next division occurs, producing four nuclei which lie grouped in the center of the oogonium (fig. 12). The oogonium then increases

to its full size; the four nuclei scatter (fig. 13), and then divide again, producing the final number of eight (fig. 14). Reduction of the chromosome number takes place in the first two divisions. The mature oogonium is surrounded by a wall of three layers, the exochiton, mesochiton, and endochiton.



FIGS. 10-18.—Fig. 10, oogonium in uninucleate stage with stalk cell; fig. 11, oogonium in which nucleus has divided; fig. 12, 4 nuclei grouped in center of oogonium; fig. 13, 4 nuclei scattered; fig. 14, third division taken place; fig. 15, 4 oospheres formed in oogonium, one abortive nucleus extruded between eggs; fig. 16, oogonium with 4 eggs; fig. 17, oblique division into 2 eggs, 6 abortive nuclei; fig. 18, longitudinal division into 2 eggs ( $\times 415$ ).

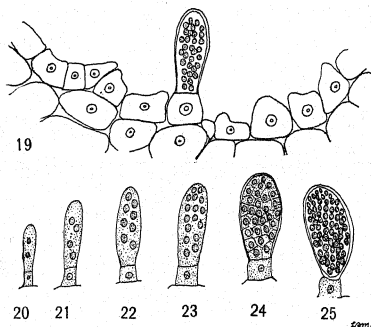
The next step is the division of the contents of the mature oogonium into eggs. The European species produces two eggs, whereas all of the material which the writer first examined produced four eggs instead of two (figs. 15, 16). This was the material collected at Redondo. Other material examined later was found to produce

in part the usual number for the European species, two eggs in each oogonium. The division was not transverse, however, but usually oblique (fig. 17) and sometimes longitudinal (fig. 18). The eggs contained one nucleus each for the most part, although binucleate eggs were found. The remaining six nuclei abort and are extruded into the mucilage which occupies the space between the eggs (figs. 15-18). The oogonia and the eggs are quite large, are very numerous in the conceptacles, and are scattered over the entire inner surface.

The antheridia are even more numerous than the oogonia, and are likewise found in all parts of the conceptacle except in the ostiole. They differ much in appearance from the antheridia of *Fucus*, because they do not as a rule grow upon paraphyses, but are produced upon a single-celled stalk just as the oogonia are, or upon a stalk of two cells. They are sometimes found branching from paraphyses, however, although this condition is not common. The antheridium initial buds from a cell of the floor of the conceptacle much as the oogonia do, except that when an oogonium is to be formed the whole upper surface of the cell becomes convex and papillate; whereas, when an antheridium is to be formed, the papillation appears at one side of the upper surface of the floor cell (fig. 19). A wall forms and cuts off the antheridial from the basal cell, following which the nucleus of the antheridial cell divides; and it now appears as a very slender, hairlike cell with two nuclei, one above the other (fig. 20). The next division forms four nuclei and reduces the chromosome number (fig. 21). The cell elongates and the four nuclei divide into eight (fig. 22). The next division produces 16 nuclei, and the antheridial cell has attained its full length (fig. 23), but grows laterally and becomes about half as wide as long. At the 32-nucleate stage evanescent walls appear (fig. 24), reminiscent of the cellular antheridia of the more primitive members of the Phaeophyceae, as *Ectocarpus*, *Cutleria*, and *Zanardinia*. The final division results in 64 nuclei (fig. 25). The ephemeral segmentation disappears in this stage, and the 64 nuclei and later the 64 sperms are free in the antheridial cavity.

The antheridium is surrounded by two walls, an inner and an outer, which separate in later stages and are readily seen. When the sperms leave the conceptacle cavity they go inclosed in this

wall. The inner wall is oval when freed from the outer, being smaller at its lower end. Antheridia were seen detached from the stalk and lying in the ostiole.



FIGS. 19-25.—Fig. 19, antheridium initials in floor of conceptacle; fig. 20, antheridium with 2 nuclei and stalk cell; fig. 21, antheridium with 4 free nuclei; fig. 22, 8-nucleate stage of antheridium; fig. 23, stage with 16 nuclei; fig. 24, 32 nuclei with delicate walls separating them; fig. 25, antheridium with 64 nuclei which will become sperms ( $\times 843$ ).

### Discussion

The apical cell of *Pelvetia fastigiata* is the typical pyramidal apical cell found in the Fucaceae, and has been described by OLT-MANNS (12) for *P. canaliculata* and by HOLTZ (6) for *P. fastigiata*. The structure of the thallus is more simple than that of most Fucaceae, and hyphae are lacking except at the base of the stele.

The filaments forming the web of the central cavity are composed of pith cells, and their walls are formed of a great number of lamellae instead of three as LE TOUZÉ (9) believed. LE TOUZÉ found the outer wall to be composed of pectocellulose and the inner wall of pectin. The lamellae are not mentioned elsewhere in the literature.

The walls of cells of the pith and cortex are perforated and there is protoplasmic connection (figs. 2, 3). LE TOUZÉ denied this but

OLTMANN (13) thinks that it is probable. All pith cells have nuclei, although LE TOUZÉ maintains that they have none. He worked on the histology of several members of the Fucaceae.

The literature up to date classes *Pelvetia* among the genera of the Fucaceae having no hair pits. However, the writer found that hair pits exist in *Pelvetia fastigiata* (fig. 4). OLTMANNS (13) states: "Fasergrübchen dürften fehlen bei *Pelvetia*." HOLTZ (6) states that conceptacles were scattered over the entire plant rather sparsely, and the writer thinks that the scattered pits found by HOLTZ were hair pits, and not conceptacles.

NORDHAUSEN (11) describes three types of hairs in conceptacles: the long colorless ones near the mouth, the branching paraphyses on which antheridia are borne, and short brown hairs. All three are found in *Pelvetia*. The short hairs seem peculiar to the immature stage and are very often found in hair pits. NORDHAUSEN believes that they crowd out the long hairs of hair pits.

*Pelvetia* has always been reported as having two eggs, except by GARDNER (5), who maintained as rare a condition in which three eggs occurred. His drawing shows the same appearance as the four-egg condition uniform for the first material investigated in this study, and occurring also in the other material (fig. 15). This number brings *Pelvetia* nearer to the *Fucus* type.

In *Pelvetia canaliculata* the contents of the oogonia divide in a transverse plane to produce two eggs, and the abortive nuclei are extruded at the equator, where they remain visible in the mucilage and finally disintegrate. HOLTZ (6) described *P. fastigiata* in the same way, while GARDNER (5) described the division as longitudinal, or sometimes oblique, and the extrusion of the abortive nuclei as occurring between the eggs. The writer has confirmed in part the views of GARDNER on this point. The division is generally oblique (fig. 17), often longitudinal when two eggs are formed (fig. 18). Only in one case out of hundreds was a transverse division found. When four eggs are formed they are arranged in tetrads. The region between the eggs is filled with mucilage in which the abortive nuclei lie.

Nowhere in the literature has mention been made of the mode of attachment of antheridia in the conceptacle in *P. fastigiata*. In-

stead of being on branched paraphyses they are borne on stalk cells like oogonia (fig. 25).

The conceptacle develops from two epidermal initials and its walls are formed from their basal segments (fig. 5). The mouth is walled by epidermal cells. The genetic layer is therefore derived entirely from the basal segments of the two initial cells. No tongue cells, such as SIMONS (15) found for *Sargassum*, are produced in *Pelvetia fastigiata*, and the basal segments are cubical instead of cup-shaped. The cortex does not enter into the conceptacle. HOLTZ believed that there was extensive disintegration in epidermal and basal cells, and that the cortex entered into conceptacle formation. BOWER (2) also believed that the cortex cells helped to form the genetic layer. HOLTZ' conclusions have been refuted by NIENBURG (10).

### Summary

1. The cell walls of the pith and cortex of *Pelvetia fastigiata* are perforated, and protoplasmic connections are evident.
2. Walls of pith cells are formed of many lamellae.
3. Hair pits exist, although not in great numbers.
4. Conceptacles are found below the fruiting tips. The lower conceptacles have no sex organs.
5. There are often four eggs in each oogonium instead of two, as usually reported. Division is not transverse but longitudinal. Binucleate eggs occur.
6. Abortive nuclei are extruded between the two or four eggs, not in the equator and outside, as in the European species of *Pelvetia*.
7. Oogonia are stalked. Antheridia are stalked like oogonia, only seldom being borne on paraphyses. Antheridia have two walls.

I am indebted to Professor C. J. CHAMBERLAIN for suggestions and criticisms.

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## STUDIES IN THE GENUS *BIDENS*. IX

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 388

EARL EDWARD SHERFF

(WITH PLATES XIV-XVI)

*Bidens fulvescens* sp. nov.—Fruticosa vel subfruticosa, caule ramisque tetragona et glabrata, demum 1.5–2.1 m. alta. Folia petiolata petiolis tenuibus usque ad 9 cm. longis, petiolo adjecto usque ad 2.2 dm. longa, pinnatim 3- vel 5-partita, juvenilia plerumque pilis numerosis fulvescentibus vel etiam ferrugineis obsita, foliolis ovato-lanceolatis vel etiam anguste oblongo-lanceolatis, apice subobtusis vel breviter acuminatis, margine serratis et ciliatis, demum plus minusve glabratis, supra valde viridibus, infra pallidioribus venulis numerosis perspicuis coloratis, basalibus plerumque petiolulatis. Capitula numerosa, corymbo-paniculata, radiata, pansa ad anthesin tantum 3.5–5 mm. alta et vix 1 cm. lata, pedicellis tenuibus, pubescentibus, saepius 0.5–2 cm. longis. Involucri bractee exteriores circ. 5, lineares, patentes vel recurvatae, tantum 1–1.5 mm. longae, minute pubescentes vel glabrae, apice subobtusae; interiores lanceolatae 3–4 mm. longae. Flores ligulati plerumque 5, flavidi, ligula late oblanceolata, apice circ. 3-dentati, tantum circ. 5 mm. longi. Achaenia linearia, nigra, obcompressa, glabra, spiraliter per 1 vel 1.5 convolutiones torta, circ. 8–11 mm. longa et 0.6–0.8 mm. lata, exaristata sed ad apicem rariter 1 vel 2 minutis setis munita.

*Otto Degener* 2515, in open woods, north slope of Mt. Kaala, Isl. of Oahu, Hawaiian Isls., February 11, 1928 (Hb. Field Mus., 3 type sheets).

*Bidens wiebkei* sp. nov.—Erecta, glabra, infra demum fruticosa supra herbacea, usque ad circ. 1 m. alta, ramis acriter tetragonis et siccis plus minusve purpurascens. Folia petiolata petiolis tenuibus plerumque 2–4.5 cm. longis, petiolo adjecto usque ad 1.3 dm. longa, 3- vel 5-partita, foliolis membranaceis lanceolatis, acriter serratis, imis 5-partitorum saepe breviter petiolulatis, terminali moderate acuminato. Capitula numerosa in inflorescentia corymboida, tenuiter pedicellata pedicellis fere glabris, radiata, pansa ad

anthesin 1-2 cm. lata et circ. 4.5-6 mm. alta. Involucri bracteae exteriores 4-6, patentes vel suberectae, lineares, apice subobtusae, tergo saepe hispidulae, 2-3 mm. longae; interiores lanceolatae, plerumque 3-4.5 mm. longae. Flores ligulati 4-6, flavidi, ligula oblongi vel late elliptico-oblancoati, apice circ. 3-denticulati, 5-9 mm. longi. Achaenia demum nigra, valde obcompressa, curvata vel saepius etiam per 1-2.5 convolutiones torta, marginata vel alata, marginibus glabris vel sparsissime erecto-setosis, nunc multo infra nunc ad corporis apicem in 1 vel 2 filiformes, remote retrorsumque hamosas usque ad 1.5 mm. longas aristas productis vel saepius aristis deficientibus; corpore faciebus glabro vel sparsim erecto-setoso, nitido, 6-8 mm. longo et 0.9-1.2 mm. lato.

*Otto Degener* and *Henry Wiebke* 3005, in scrub vegetation, upper part of Halawaiki Gulch, Isl. Molokai, Hawaiian Isls., June 21, 1928 (3 type sheets, Hb. Field Mus.: cotypes, Hb. Berl.; Hb. Boiss.; Hb. Brit. Mus.; Hb. Mun.; Hb. Mus. Vienna; Hb. N.Y. Bot. Gard.). Pl. XIV.

A species allied to *Bidens sandvicensis* Less., a species unknown in its normal state except from the Islands of Kauai, Oahu, Maui, and Hawaii. *B. sandvicensis* var. *caduca* Sherff was originally described by HILLEBRAND from Molokai, without stating the exact locality. Apparently the var. *caduca* is widely different from *B. wiebkei* in having shallower "serratures and cusps, . . . achenes not margined . . ." and in having achenes straight or only lightly curved. Some of the many specimens of *B. wiebkei* examined have the achenes nearly all exaristate, but a few aristate with one or two aristae at the very apex. Others have 1 or 2 aristae rather commonly, these coming out about one-fifth to one-third the way down the sides, and being continuous with the conspicuously flattened achenial margins. The heads are more numerous than in *B. sandvicensis* proper, and when in fruit differ noticeably because of the more or less twisted and bent achenes.

The species is named in honor of *Mr. Henry Wiebke*, who, in collaboration with *Professor Degener* of the University of Hawaii, has done much valuable work in collecting the native Hawaiian plants for taxonomic study.

*Bidens coartata* sp. nov.—Perennis, infra fruticosa,  $\pm$  5 dm. alta, ramosa, caule ramisque tetragonis, glabris. Folia petiolata petiolis

tenuibus usque ad 6 cm. longis, petiolo adjecto usque ad 1.5 dm. longa, pinnatim 3-5-partita, foliolis ovatis vel lanceolatis, membranaceis, acriter dentibus calloso-apiculatis serratis, glabris ac eciliatis, imis saepe ad marginem inferiorem plus minusve irregulariter divisus, terminali apice breviter acuminato. Capitula subnumerosa et saepe in inflorescentia densa subcorymboideaque coarctata, radiata, pansa ad anthesin circ. 1.5 cm. lata et 6-8 mm. alta. Involucri bracteae glabrae vel glabratae, exteriores circ. 5-7, lineares, apice subobtusae, circ. 3 mm. longae; interiores lanceolatae paulo longiores. Flores ligulati plerumque 5, flavidi, ligula late ob lanceolato-elliptici, apice subintegri, circ. 7-10 mm. longi. Achaenia anguste linearia, nigra, exalata, plana, recta vel moderate torta, faciebus glabrata et leviter paucistriata, marginibus sparsim suberecto-hispida, apice aegre spinulosa sed exaristata, corpore 7-10 mm. longa et 0.6-1 mm. lata, demum paleas manifeste superantia.

*Otto Degener 2677b*, sunny slopes from Woodlawn along east rim of Manoa Valley toward Mt. Olympus, Isl. of Oahu, Hawaiian Isls., February 28, 1928 (2 type sheets, Hb. Field Mus.: cotypes, Hb. Berl.; Hb. Brit. Mus.; Hb. N.Y. Bot. Gard.); *idem 2676*, two-thirds the way up Mt. Olympus by way of Pauoa Flats, Isl. Oahu, February 25, 1928 (Hb. Brit. Mus.; Hb. Field Mus.; Hb. N.Y. Bot. Gard.).

***Bidens salicoides* sp. nov.**—Fruticosa, erecta, glabra, ramosa,  $\pm 6$  dm. alta, caule ramisque plus minusve tetragonis. Folia petiolata petiolis tenuibus usque ad 5 cm. longis, petiolo adjecto usque ad circ. 1.5 dm. longa, principalia pinnatim sed saepe irregulariter 2-5-partita, foliolis moderate vel late linearibus ac circumambitu foliis non nullorum specierum *Salicis* nonnihil similibus, integris vel interdum paucidentatis, marginibus saepe parce revolutis, membranaceis, terminali usque ad 8 cm. longo et 12 mm. lato, lateralibus plerumque minoribus et sessilibus vel imis subpetiolulatis; summa nunc indivisa, nunc ternata. Capitula subcorymboidea, radiata, pansa ad anthesin  $\pm 1.5$  cm. lata et circ. 6-8 mm. alta, graciliter pedicellata pedicellis 1-4 cm. longis. Involucrum glabratum, bracteis exterioribus circ. 6-7, anguste linearibus, apice calloso subacutis, costa mediana atris, circ. 3-4 mm. longis, quam interioribus lanceolato-ovatis paulo brevioribus. Flores ligulati 3 vel 4, forsitan interdum 5, flavidi, lineari-elliptici, apice obsolete denticulati, 1.2-1.4 cm.

longi. Achaenia linearia, plana, plumbeo-atra, exalata, recta vel moderate torta, plerumque glabra, faciebus obscure sulcata (omnino circ. 16 sulcis), corpore 7-10 mm. longa, apice ipso nunc 1- vel 2-aristata aristis nudis usque ad 1 mm. longis, nunc calvis.

Henry Wiebke 3084, arid region, East Ohia, Isl. Molokai, July 17, 1928 (Hb. Field Mus., 3 type sheets: cotypes, Hb. Berl.; Hb. N.Y. Bot. Gard.). Pl. XV.

The five specimens studied had come from a single plant, the only one found. Professor DEGENER, to whose kindness I am indebted for the privilege of examining the material, had suspected that this might be "HILLEBRAND'S lost variety"; i.e., *Campylothecha sandvicensis* var.  $\beta$ , Hillebr. Fl. Hawaiian Isls. 214. 1888. HILLEBRAND'S plant, renamed by me *Bidens sandvicensis* var. *caduca* (BOT. GAZ. 85:7. 1928), came from an unknown locality on the Island of Molokai and was described in terms that might indeed apply to our plant. However, from a study of HILLEBRAND'S various determinations of Hawaiian Island *Bidens* material to be found at Gray Herbarium and in the British Museum of Natural History, it is difficult to believe that HILLEBRAND would have referred this plant to the species *sandvicensis*. Apparently the identity of his variety must remain obscure until an authentic specimen labeled by him can be located.

Since the present plant is clearly separate from *B. sandvicensis* and requires a distinct specific name, and since it is impossible to utilize the name *caduca*, I have chosen *salicoides*. This name is suggested by the illusory resemblance of the herbarium specimens to specimens of various species of *Salix*, a resemblance arising from the *salicoid* outline of the simple leaves or, in the case of the much more numerous compound leaves, of the leaflets.

*Bidens populifolia* sp. nov.—Herba e radice (ut videtur) annua, usque ad 8 dm. alta, glabra, caule tetragono, subsimplici vel etiam valde ramoso, non robusto. Folia petiolata petiolis tenuibus nunc 1.5-6 cm. nunc 6-10 cm. longis, petiolo adjecto usque ad 2 dm. longa, membranacea, pallidiora, vix ciliata, serrata dentibus rotundatis obtuseque calloso-apiculatis, plerumque indivisa, circumambitu ovato-cordata vel deltoideo-cordata, basi saepe tantum subcordata vel etiam truncata obliquave, apice nunc subobtusula nunc acuta vel

rarius plus minusve subacuminata; rariter (et plus minusve irregulariter) pauca 2-3-partita, foliolo terminali late rhomboideo-lanceolato vel ovato-lanceolato vel rotundato, lateralibus obliquis et rhomboideo-ovatis. Capitula non numerosa, in inflorescentia corymbiformi disposita, radiata, pedicellata pedicellis tenuibus usque ad 5 cm. longis, pansa ad anthesin circ. 3 cm. lata et 7-9 mm. alta. Involucri bracteae valde dissimiles, exteriores 5-7, patenti-reflexae, glabrae, nunc late oblongo-lineares nunc spathulato-obovatae apice obtusae vel rotundatae, 1.5-3.5 mm. longae; interiores lanceolatae, apicem versus minute pubescentes, 5-6.5 mm. longae. Flores ligulati 6-8 (rariter tantum 5), flavidi, ligula oblongo-lineares vel oblanceolati, apice plerumque 3-dentati, 1.2-1.5 cm. longi. Achaenia anguste linearia, recta, exalata, subnigra, obcompressa, unica facie obsolete circ. 8-striata, margine sparsim erecteque ciliata, corpore 7-12 mm. longa et 0.8-1.1 mm. lata et paleas apice rufescentes paulo demum saepe excedentia, apice plerumque biaristata aristis tenuibus, supra retrorsum infra antrorsum hamosis, usque ad 1 mm. longis.

Otto Degener, Ross S. Bean, D. Le Roy Topping, and Anthony Apo 2514, growing with *Pandanus* and stunted *Metrosideros*, grassy slope back of small Hawaiian church on east side of Kahana Valley, Isl. Oahu, Hawaiian Isls., February 12, 1928 (Hb. Field Mus., 4 type sheets: cotypes, Hb. Berl.; Hb. Boiss.; Hb. Brit. Mus.; Hb. Gray; Hb. Kew; Hb. Mo. Bot. Gard.; Hb. Mun.; Hb. Mus. Vienna, etc.); Ross S. Bean 2322, sunny, rocky ridge, foot of left ridge of Kahana Valley, Isl. Oahu, Hawaiian Isls., January 1, 1928 (Hb. Field Mus.; Hb. Kew). Pl. XVI.

This species is characterized by the very distinct *Populus* aspect of its foliage, the resemblance to the foliage of *P. deltoides* Marsh, for example, being at times very striking. HILLEBRAND (Fl. Hawaii. Isls. 215. 1888) appears not to have known this plant. Under *Campylotheca macrocarpa* he lists the (to *B. populifolia*) faintly similar var. *ovatifolia*, transferred by him from varietal rank under *Bidens sandwicensis* Less., where ASA GRAY had originally placed it. He then gives a detailed description of mature plants, a description which could not have come from the single, very immature type of the var. *ovatifolia* (U.S.). The true var. *ovatifolia*, recently placed by me (Bot. Gaz. 85:7. 1928) under *Bidens macrocarpa*, has sharply

serrate leaves, while *B. populifolia* has round and obtuse teeth. It is clear from HILLEBRAND's text that, the synonym var. *ovatifolia* Gray being excluded, his treatment applied to the herbaceous forms of *Bidens asymmetrica* (Lévl.) Sherff, found upon Oahu. In fact, we may note that one able student of the Hawaiian flora, JOSEPH ROCK, had labeled a cotype specimen of *B. asymmetrica* (Faurie 960, Par.) with the equation: "*Coreopsis macrocarpa* Hbd. var.  $\beta$  Hbd. = *Lipochaeta asymmetrica* Lévl. (teste) Rock." (the latter name being a synonym for *Bidens asymmetrica*). A consideration of these herbaceous forms of *B. asymmetrica* ("Achenes . . . often spirally twisted"), however, shows that they too have little in common with the species here named *B. populifolia*.<sup>1</sup>

**BIDENS MAUIENSIS cuneatoides** var. nov.—A specie foliis nunc indivisis nunc tripartitis laminis segmentisve lanceolatis vel ovatis vel subrhomboideis vel etiam late cuneato-spathulatis, habitu *Bidentii cuneatae* non dissimilis differt.

Otto Degener and Henry Wiebke 2261, arid aeolian deposits southeast of Wailuku, Isl. of Maui, Hawaiian Isls., Jul. 7, 1927 (Hb. Berl.; Hb. Boiss.; Hb. Field Mus., 2 sheets; Kew; Mun.); *idem* 2680, on barren, aeolian deposits near Wailuku, Isl. of Maui, Hawaiian Isls., July 9, 1927 (type in Hb. Field Mus.: cotypes in Hb. Berl.; Hb. Boiss.; Hb. Brit. Mus.; Hb. Kew; Hb. N.Y. Bot. Gard.); H. Mann and W. T. Brigham 372, Isthmus of Maui (Hb. Bish. Mus.; Hb. Brit. Mus.; Hb. Cornell Univ.; Hb. Deless.; Hb. Field Mus., 2 sheets; Hb. Gray; Hb. Kew; Hb. Mo. Bot. Gard., etc.).

The type material of *Bidens mauiensis* (*United States Southern Pacific Exploring Expedition* under Capt. Wilkes, sandy or dry hills near the coast, Island of Maui, Hawaiian Islands, 1838-1842; Hb. Gray) had the leaves, except for one small branchlet apparently somewhat ignored by ASA GRAY in writing his original description, rather well dissected, with linear segments. The duplicate sheet in the Torrey Herbarium (Hb. N.Y. Bot. Gard.) has, at the right, one specimen with some leaves tripartite and some undivided, the blades or their segments more or less rhombic-ovate and somewhat suggestive of those of *Bidens cuneata* Sherff. Doubtless had GRAY seen this last specimen he would have presented a different or an addition-

<sup>1</sup> It is a pleasure to acknowledge my indebtedness to Messrs. Bean and Degener, also their associates, for making special trips to the type locality and securing a large representation of material for my study.

al treatment. In any case, we are fortunate in having at hand two new and excellent series of specimens collected in July, 1927, by *Degener* and *Wiebke* in the type region. Nos. 2678 and 2679 by these collectors are of the species as described by GRAY. Their nos. 2261 and 2680 have the leaves simple or merely tripartite. These latter thus match the ignored or at least undescribed specimens collected together with GRAY's type of the species proper. They are matched in turn by *Mann* and *Brigham* 372. The specimens of this last number at Delessert Herbarium and at Bishop Museum have the leaves mostly simple, but elsewhere they are mostly tripartite.

The pronounced difference between the two types of foliage makes it appear worth while to treat the plants with the simple or tripartite leaves as varietally distinct.

*BIDENS MAUIENSIS forbesiana* var. nov.—Ex characteribus maximam partem var. *lanaiensi* similis sed foliis plerumque indivisis rarius tripartitis differt.

G. C. Munro 451, Maunalei, Isl. of Lanai, Hawaiian Isls., April 19, 1915 (type in Hb. Bish. Mus.).<sup>2</sup>

This and the next following were included by me in a former paper (BOT. GAZ. 80:381. 1925) among the plants referred to *B. mauiensis* var. *lanaiensis*. The considerations already referred to, however, for the var. *cuneatoides* make it appear similarly preferable here to segregate from the var. *lanaiensis* the two types of foliage which differ in not being finely dissected.

The name *forbesiana* alludes to Professor CHARLES N. FORBES, who, before his death, had studied the type plant and regarded it as typifying a new variety.

*BIDENS MAUIENSIS media* var. nov.—Ex characteribus maximam partem var. *lanaiensi* similis sed foliis minus decomposita; a var. *forbesiana* foliis non plerumque indivisis differt.

G. C. Munro 450, Maunalei, Isl. of Lanai, Hawaiian Isls., April 19, 1915 (type in Hb. Field Mus.: cotype in Hb. Bish. Mus.).

<sup>2</sup> In view of the present comparative inaccessibility of much of the material in the Bishop Museum (Honolulu), it may be remarked that this and some 2000 other important specimens of *Bidens*, *Coreopsis*, *Cosmos*, *Isostigma*, *Taraxacum*, *Xanthium*, and Compositae generally are represented by the writer's photographs, a duplicate set of which is in the Herbarium of Field Museum of Natural History (Chicago).

*BIDENS BIPINNATA* L. Sp. Pl. 832. 1753; *B. fervida* Nocca in Fischer Cat. Gorenki edit. II, 37. 1812 (*nomen*); *B. myrrhidifolia* Tausch, Flora 19:394. 1836; *B. cicutaefolia* Tausch, *loc. cit.* 395; *B. elongata* Tausch, *loc. cit.* (*ex descript. etc.*); *B. tenuifolia* Tausch, *loc. cit.*; *B. decomposita* Wall. ex DC. Prodr. 5:602. 1836; *Coreopsis corymbifolia* Ham. in Wall. ex DC.<sup>3</sup> *loc. cit.*; *Bidens pilosa* var. *decomposita* (Wall. ex DC.) J. D. Hook. Fl. Brit. Ind. 3:309. 1881; *B. kotschyi* Schz. Bip. ex Walp. Rep. 6:168. 1846; *B. kotschiana* Schz. Bip. *loc. cit.*; *Kerneria bipinnata* (L.) Godr. et Gren. Fl. Fr. 2:169. 1850; *B. pilosa* var. *bipinnata* (L.) J. D. Hook. *loc. cit.*; *B. pilosa* var. *decomposita* (Wall. ex DC.) J. D. Hook. *loc. cit.*<sup>4</sup>

FISCHER (*loc. cit.*) cited "*B. fervida* Noccoa," which of course was erroneous as to the spelling of NOCCA's name. With FISCHER, *B. fervida* amounts to a name (*nomen*) only. In NOCCA's Synonymia Plant. Hort. Bot. Ticinensis (1804), *B. fervida* is not given. NOCCA's many references there to LAMARCK's Encyclopaedia, however, show that he was entirely familiar with that work, and it was in that work that *Bidens fervida* Lam. was published. The real *B. fervida* Lam. was *Spilanthes oleracea* L. and is generally regarded as such (*cf.* Moore, Proc. Amer. Acad. 42:530. 1907). NOCCA's material does not appear to have become widely distributed in European herbaria, but the Ledebour Herbarium (Hb. Petrop.) has an old folder with two unmounted specimens which are without doubt authentic. They are in fruit and in excellent condition. Both are *B. bipinnata* L.

TAUSCH (*loc. cit.*) had sought to distinguish among five forms cultivated in gardens under the name *B. bipinnata*. He described each form separately, retaining one as *B. bipinnata* and creating new names for the other four. *B. cicutaefolia* Tausch and *B. myrrhidifolia* Tausch each are represented by one of his own cultivated specimens (Hb. Univ. Leips.) and are seen to be purely *B. bipinnata*.<sup>5</sup>

<sup>3</sup> DECANDOLLE cited Wallich's Catalogue, but the name there given is "*Coreopsis ? corindifolia* Ham."

<sup>4</sup> It being my intention at this time only to discuss the status of TAUSCH's proposed names and to place on record certain scattered but important observations, no attempt is made here to present an exhaustive synonymy of *B. bipinnata* or to discuss all of the synonyms given.

<sup>5</sup> TAUSCH unfortunately cited as a synonym for his *B. cicutaefolia* the name *Chrysanthemum chinense*, etc., Plukenet Phytograph. pl. 22, fig. 4, 1691. PLUKENET's plant, however, was *B. chinense* (L.) Willd., as stated by me in a former paper (Bot.



The meager description of *B. tenuifolia* Tausch matches *B. bipinnata* as far as it goes. A plate cited by TAUSCH, "*Chrysanthemum cannabinum* etc. Moris. Hist. 3<sup>VI</sup>: tab. 7, fig. 23," is of *B. bipinnata* and is so treated by DECANDOLLE (Prodr. 5:603. 1836) and O. E. SCHULZ (Engler Bot. Jahrb. 50, Suppl.:183. 1914). At Leipsic I sought, in 1924, to find further evidence in the form of authentic specimens of *B. tenuifolia* Tausch, but failed entirely.

*B. elongata* Tausch appears likewise unrepresented by an authentic specimen today, but the description given by TAUSCH, as also his context, leaves no doubt as to the identity of *B. elongata* with *B. bipinnata*.

HOOKE (loc. cit.) treated *B. pilosa* L. as being made up of three varieties, viz. *pilosa* proper, *bipinnata*, and *decomposita*. Taxonomists very generally, however, have refused to accept such treatment. *B. pilosa* and *B. bipinnata*, while sometimes approaching each other, are usually quite different and easily distinguished.

*B. decomposita* Wall. ex DC. was founded upon WALLICH's Catalogue no. 3188 (DC. distrib. no. 298). A fine specimen of this number, from KUNTH's herbarium, is still extant (Hb. Berl.). This is purely *B. bipinnata* L., and, in fact, had been so determined in 1911 by O. E. SCHULZ. Similarly other specimens of this number (Hb. Prodr. in Hb. Deless.; Hb. Kew) are either normal *B. bipinnata* or represent slight and insignificant variations from *B. bipinnata*.

BIDENS PILOSA var. MINOR (Bl.) Sherff, Bot. Gaz. 80:387. 1925; *Coreopsis leucorrhiza* Lour. Fl. Coch. edit. I:508. 1790 (ex descript. et patria); *C. leucorrhiza* Lour. loc. cit. edit. II:622. 1793 (ex descript. et patria); *Bidens hispida* H. B. K. Nov. Gen. et Sp. 4:186 (237). 1820; *B. leucorrhiza* (Lour.) DC. Prodr. 5:605. 1836; *B. andicola* var.  $\beta$ , Wedd. Chlor. And. 1:70. 1855 (ex synonym. H. B. K.).—DECANDOLLE (loc. cit.) treated LOUREIRO's *Coreopsis leucorrhiza* with misgivings, placing it under *Bidens* with an interrogation and listing it among the species not well enough known. FORBES and HEMSLEY (Jour. Linn. Soc. 23:435. 1888) declared LOUREIRO's plant to have

Gaz. 61:499. 1916). A consideration of TAUSCH's entire context, much of which was inaccessible when writing the former paper, shows that TAUSCH's name *B. cicutaeifolia* must rest upon the real plants studied by him, also their published description, and that the reference to PLUKENET must be ignored.

been "insufficiently described for recognition."<sup>6</sup> Reference to LOUREIRO's rather full description shows that the plant described grew in fields near Canton, China. The leaves were 5-partite, their leaflets lanceolate. The flowers were yellow and the heads had 6 rays, these neuter and with ovate ligules. The achenes were 3-aristate, with the aristae retrorsely barbed. There is only one *Bidens* known in the vicinity of Canton which fits this description. That is *B. pilosa* var. *minor* (Bl.) Sherff.<sup>7</sup> In certain herbaria are a number of specimens from places near to the type locality (Hongkong, Hainan, etc.), among them the following:

*Hance* 299, Hongkong (Hb. Gray); *Dr. Aug. Henry* 75, Hongkong (Hb. Mo. Bot. Gard.); *idem* 8769, Isl. of Hainan, November, 1889 (Hb. Gray); *C. O. Levine* 278, Isl. of Honam, Kwang Tung Province, October 1, 1917 (Hb. Mo. Bot. Gard.); *C. Wright*, Hongkong, 1853-1858 (Hb. Gray).

In a former article (BOT. GAZ. 61:497. 1916), I stated the reasons for reducing *B. hispida* H.B.K. to synonymy under *B. pilosa* L. The type of *B. hispida* (Hb. Par.) had been described as having the florets not yet open. At the former writing I had been impressed with the fact that several more mature specimens matching the type of *B. hispida* had been collected by various botanists, and that these tended to have minute, yellowish-white rays. In 1924 it was my good fortune to find a better developed cotype specimen in the Willdenow Herbarium, no. 15022, fol. 3 (Hb. Berl.). The one small flowering head had three or four minute rays and thus revealed definitely the identity of *B. hispida* with the minutely radiate form of *B. pilosa* that passes as var. *minor*.

BIDENS PILOSA L. Sp. Pl. 832. 1753; *B. decussata* Pav. ex DC. Prodr. 5:599. 1836.—In the Prodrômus Herbarium (Hb. Deless.) are five sheets to represent *B. hispida* H.B.K. These all bear only ordinary *B. pilosa* L. One has an old label saying "*B. decussata* Perou Pavon." and, being clearly the basis of the name *B. decussata* Pav. ex DC., enables us at once to reduce this name to synonymy with *B. pilosa* L.

<sup>6</sup> For many data regarding LOUREIRO and his work, *vide* American Botanist 25: 129. 1919; *ibid.* 26: 28. 1920.

<sup>7</sup> *B. pilosa* var. *radiata* Schz. Bip. is found in that vicinity but its rays are more of a whitish or yellowish-white color, not a pure yellow as described by LOUREIRO.

*B. DURANGINENSIS* Sherff, BOT. GAZ. 70:90, Pl. XI. 1920; *B. anthriscoides* var. *angustiloba* DC. Prodr. 5:601. 1836.—DECAN-DOLLE founded his *B. anthriscoides* var. *angustiloba* upon *Berlandier* 875, collected at the City of Mexico. His type is still extant (Hb. Deless.) and is found to be inseparable from *B. duranginensis*, a species much closer to *B. pilosa* L. than to *B. anthriscoides* DC.

*Bidens musoziana* nom. nov.; *Coreopsis arenicola* S. L. Moore, Jour. Linn. Soc. 37:170. 1905; *Bidens arenicola* (S. L. Moore) Sherff, BOT. GAZ. 59:309. 1915; *non* Gandoger Fl. Lyon. 122. 1875.—This species is here renamed<sup>8</sup> to avoid any possible confusion with the ill-advised name *B. arenicola* Gand. The first cited specimen of GANDOGGER's species was one from Arnas (Rhône), France. GANDOGGER cited also the synonym *B. arenaria* of his own mss. In herbaria we have at least four good specimens of GANDOGGER's own collecting in the vicinity of Lyons and labeled by him *B. arenaria* Gdgr. One, without a number, was collected at Arnas, Rhône, September, 1872 (Hb. Mus. Vienna). A second and third, *Gandoger* 967, were collected at Arnas, August 11, 1869 (Hb. Mo. Bot. Gard.; Hb. Univ. Vienna). A fourth, *Gandoger* 599, Anse, Rhône, was collected August 6, 1866 (Hb. Kew). These all are merely simple-leaved forms of *B. tripartita* L.

*BIDENS CERNUA* L. Sp. Pl. 832. 1753; *B. cusickii* Greene, Pittonia 4:259. 1901.—In a former article (BOT. GAZ. 61:503. 1916), I discussed the large number of field forms which E. L. GREENE had segregated from *B. cernua* L. Some of these were reduced at that time to synonymy with *B. cernua*. *B. cusickii*, however, was left untreated until further specimens could be examined.

*B. cusickii* was founded by GREENE upon Wm. C. *Cusick* 1768, tules of Grande Ronde Valley, Oregon, August, 1897. Specimens of the type number are in Field Museum and at Kew. These both are slightly atypic for *B. cernua* and suggest very faintly an approach toward *B. laevis* (L.) B.S.P. On the whole, however, both the foliage and the floral characters are too close to those of *B. cernua* to permit any segregation as a distinct species.

<sup>8</sup> The type was collected at Musozi, Uganda Protectorate. The name was spelled by the collector, A. G. *Bagshawe*, Nuzi. By the Century Atlas (259, pl. 112. 1911) it is spelled Msozi. SPENCER MOORE's spelling is the one adopted here.

*BIDENS PILOSA* var. *ALAUSENSIS* (H.B.K.) Sherff, BOT. GAZ. 81: 35. 1926; *B. diversifolia* Willd. ex DC. Prodr. 5:602. 1836.—WILLDENOW's original sheet of his own *B. diversifolia* is in the Willdenow Herbarium (Hb. Berl.). It bears two specimens. One is referable to *B. pilosa* var. *alausensis* (H.B.K.) Sherff, but the other appears to belong to *B. andicola* H.B.K. It was probably because of this very unlikeness between the two specimens that WILLDENOW used the name *diversifolia*. The case is evidently one of improper commixture of specimens at the time of collecting or mounting. Fortunately, the very definite status of the names *alausensis* and *andicola* permits our ignoring the name *B. diversifolia* Willd. ex DC.

*BIDENS RADIATA* Thuill. Fl. Par. edit. II: 422. 1799; *B. foliosa* Willd. Enum. Hort. Berol. Suppl. 56. 1813 (*nomen subnudum*); etiam Loudon Hort. Brit. 335. 1830.—WILLDENOW's *B. foliosa* was listed as an "annual. Growing always in the open." It was listed later by LINK (Enum. Pl. Hort. Berol. 305. 1822) and by J. C. LOUDON (*loc. cit.*), the latter giving a more extended description and evidently having seen some authentic material. The type in the Willdenow Herbarium at Berlin is Hb. Willd. 15020-5. ASCHERSON, in 1869, had labeled it "*B. radiata* Thuill. fol. indivis.

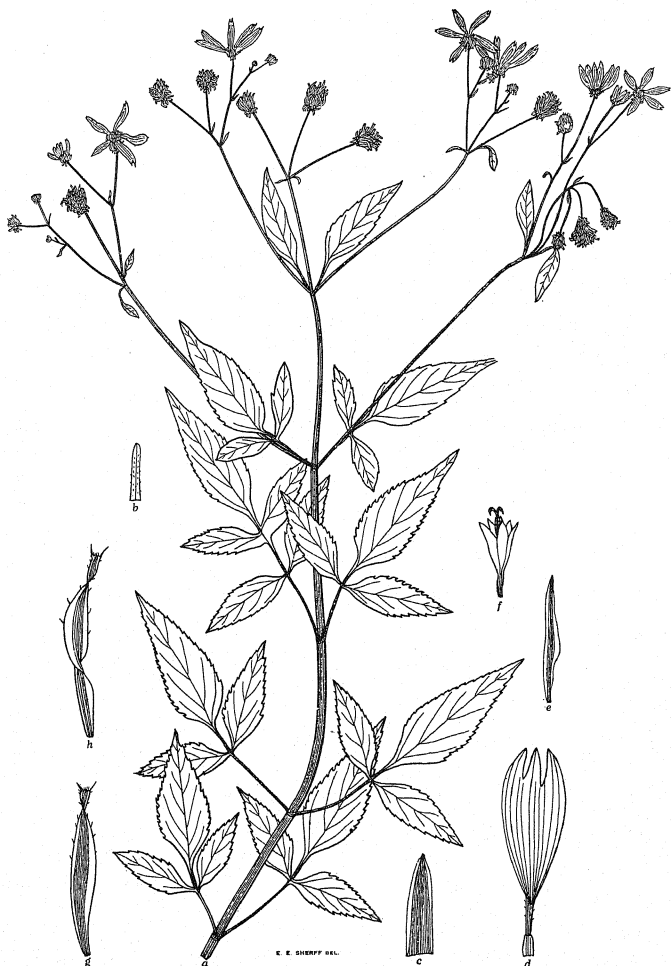
" A duplicate sheet in the Berlin Herbarium bears three plants, all with leaves likewise simple. The achenes on all four plants are typical for *B. radiata*, with which the name *B. foliosa* must be considered synonymous.

The reason for WILLDENOW's name *B. foliosa* is hardly apparent, until one examines the duplicate specimen in Vienna (Hb. Mus. Vienna). This is a spray with gigantic leaves, which measure up to more than 2.1 dm. long. One leaf is tripartite, with the terminal leaflet oblong-lanceolate. The others are simple.

This mostly simple-leaved form of *B. radiata* is much rarer than are simple-leaved forms of the sister species, *B. tripartita* L.

*BIDENS CORONATA* var. *tenuiloba* (Gray) comb. nov.; *Coreopsis trichosperma* var. *tenuiloba* Gray Syn. Fl. Nl. Amer. 1:295. 1884; *Bidens trichosperma* var. *tenuiloba* (Gray) Britt., Bull. Torr. Bot. Club 20:281. 1893; *B. trichosperma* var. *tenuifolia* (Gray) Britt. ex Farw., Ann. Rept. Comm. Parks and Blvds. Detroit 11:92. 1900.

Frequently, where many specimens are examined in the field, the var. *tenuiloba* is found to pass over into the species proper. The



E. E. SHERFF DEL.

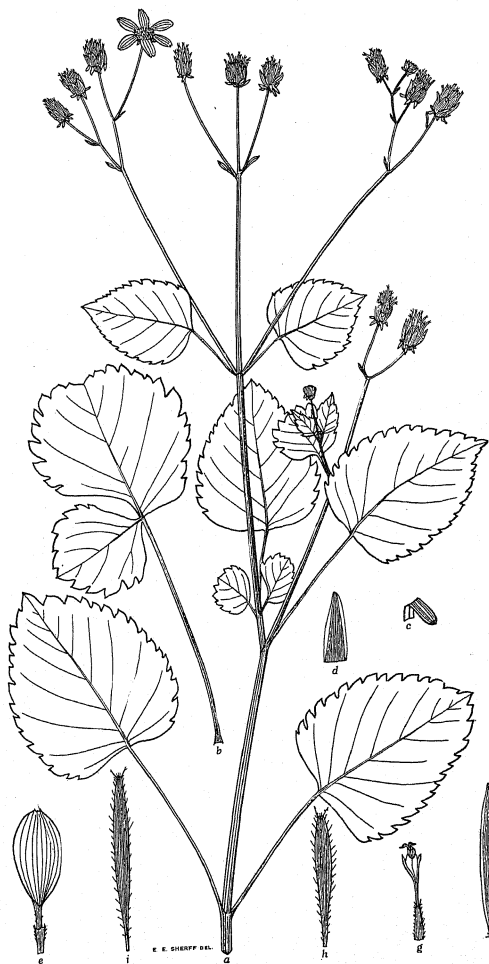
SHERFF on BIDENS



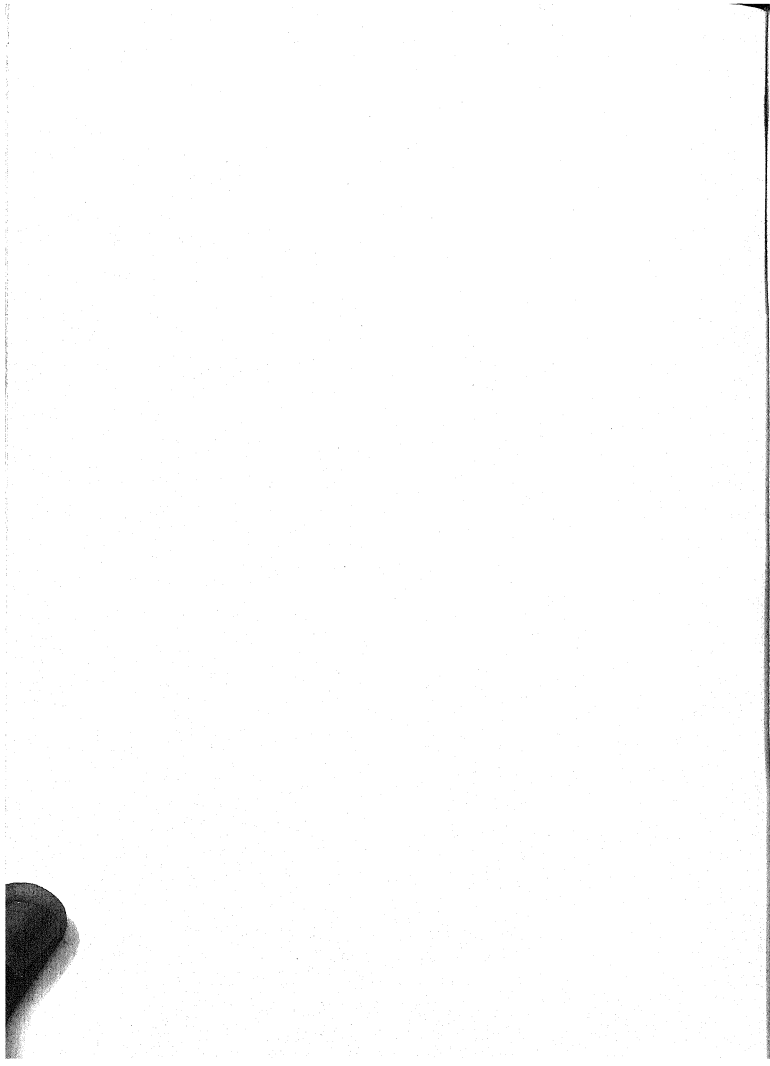








SHERREE on BIDENS



more finely divided foliage appears to be the most pronounced diagnostic character, but even this is inconstant. Rarely the achenes are exceedingly diminutive and then a strong approach is made to *Bidens mitis* (Michx.) Sherff, as noticed by GRAY (*loc. cit.*).

FARWELL's plant, not seen by me, was referred with an interrogation to this variety. It was described as "a peculiar form found on Belle Isle," a pleasure island at Detroit, Michigan.

CHICAGO NORMAL COLLEGE  
CHICAGO, ILL.

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## EXPLANATION OF PLATES XIV-XVI

### PLATE XIV

*Bidens wiebkii*: *a*, flowering and fruiting branch,  $\times 0.6$ ; *b*, exterior involucre bract,  $\times 3.6$ ; *c*, interior involucre bract,  $\times 3.6$ ; *d*, ligulate floret,  $\times 3$ ; *e*, palea,  $\times 3.6$ ; *f*, disc floret,  $\times 6$ ; *g*, *h*, achenes,  $\times 3.6$ ; all from Degener and Wiebke 3005, cotype in Hb. Mus. Vienna.

### PLATE XV

*Bidens salicoides*: *a*, flowering and fruiting branch,  $\times 0.6$ ; *b*, exterior involucre bract,  $\times 3.6$ ; *c*, interior involucre bract,  $\times 3.6$ ; *d*, ligulate floret,  $\times 3$ ; *e*, palea,  $\times 3.6$ ; *f*, disc floret,  $\times 6$ ; *g*, *h*, exterior and interior achenes,  $\times 3.6$ ; all from Wiebke 3084 (*a* from cotype in Hb. Berl. and the rest from 1st type sheet in Hb. Field Mus.).

### PLATE XVI

*Bidens populifolia*: *a*, main portion of younger plant,  $\times 0.6$ ; *b*, leaf from lower part of same,  $\times 0.6$ ; *c*, exterior involucre bract,  $\times 3.6$ ; *d*, interior involucre bract,  $\times 3.6$ ; *e*, ligulate floret,  $\times 1.8$ ; *f*, palea,  $\times 3.6$ ; *g*, disc floret,  $\times 6$ ; *h*, *i*, achenes,  $\times 3.6$ ; all from Degener, Bean, Topping, and Apo 2514, cotype in Hb. Boiss.

# RELATION OF PHOSPHORUS CONTENT TO SHRIVELING OF WALNUT KERNELS<sup>1</sup>

A. R. C. HAAS AND L. D. BATCHELOR

(WITH TWO FIGURES)

## Introduction

It is a matter of common observation that mature walnut kernels may take on various shades of amber color, and in addition show different degrees of plumpness. They may be only partially shriveled, or may be completely so, as illustrated by fig. 1. The present

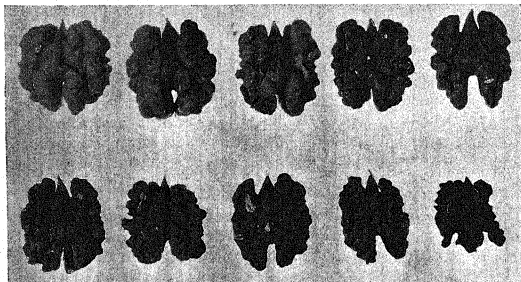


FIG. 1.—Various degrees of shriveling in walnut kernels, progressively from left to right in both rows; normal plump kernel is one extreme and “blank” kernel is the other.

standards by which walnuts are marketed are based largely on the color of the kernel and also upon the number that are free from any shriveled condition.

The walnut kernel in the early stages of development is filled with liquid, as is illustrated by fig. 2. Later gel formation takes place along the surfaces within the kernel. While this gel formation is taking place the shell becomes hardened, first at the apex and along

<sup>1</sup> Paper no. 191, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

the suture, and then generally throughout the entire shell. In the case of most varieties grown in southern California, the contents of the kernel have jelled considerably by late June and early July, and the entire shell has become hardened; toward the end of July or early in August the kernels have become solid. In September and October the husks should separate readily from the shell and the mature walnuts have become ready to be harvested and cured. When the nuts are graded in the packing houses some may be extremely light in weight, and may be removed by the use of air suc-

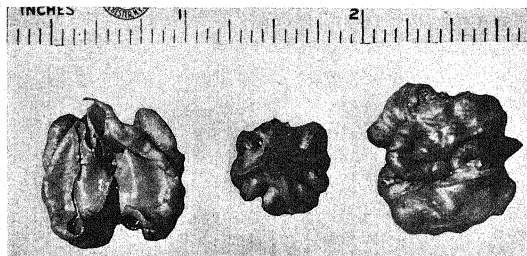


FIG. 2.—Interior and exterior view of walnut kernels when contents are still liquid

tion. The contents of such walnuts are commonly shriveled kernels or "blanks."

The view is commonly held that the shriveling of walnut kernels must take place, if at all, while the kernel is in the liquid condition, and that it may be a direct result of a shortage of moisture for the enlargement of the growing kernel while in this condition. The writers have found no evidence for this assumption; and, by using the content of phosphorus as a criterion of the age of kernels, have come to the conclusion that shriveling of the kernels takes place after they have passed the liquid condition, have jelled, and should be approaching the solid stage. The water requirement of the trees during the hot months of July, August, and early September is no doubt enormous, and during this period the soil moisture supply may fall

below the wilting point of the trees.<sup>2</sup> In the competition for available water the leaves may perhaps draw some of their supply at the expense of the kernel. This, in addition to direct losses of water from the husk, may divert to the husk part of the water supply that normally would go to the kernel. Such action is the greater especially in districts that experience high temperature and low humidity. It may also be more extreme on trees that have many of the nuts exposed to the direct heat of the sun. A combination of all of these unfavorable factors may bring about shriveling of the kernels and may thereby reduce the value of the crop.

### Experimentation

Walnut kernels collected on September 17 were placed in an oven at 65° C. After several weeks the kernel coat became light russet, but the meat remained white and there was no perceptible shrinkage. Kernels that were collected in July, just past the gel stage, however, not only shriveled badly but became dark colored when dried at 65° C. The crucial stage in the development of the kernel appears to come just before the time of solidification. If during this period the withdrawal of water is excessive, and the intake or translocation of carbohydrate material is reduced, a shriveled condition of the kernel may be expected. The intake of carbohydrate material by the kernels may be reduced by drought or by aphid infestation.

Kernels were secured from walnut trees on July 13 in Riverside, California, and again from the same trees on September 23, for observation of changes in the fresh weight, dry weight, and ash content during this important period. Table I shows the results obtained.

The kernels of the seedling and Payne walnuts obtained in July and in September show in two cases a loss in fresh weight with the increased age of the kernel, while in the case of Placentia kernels there was an increase in weight. During this period there was an enormous increase in the dry weight of all the kernels, and the ash about doubled its value. The ash as a percentage of the fresh weight increased about twice its value, except in the case of Placentia kernels, where the increase was not so great. The ash as a percentage

<sup>2</sup> BATCHELOR, L. D., and REED, H. S., The seasonal variation of the soil moisture in a walnut grove in relation to the hygroscopic coefficient. Calif. Agric. Exp. Sta., Tech. Paper 10. 1-31. 1923.

of the dry matter in July ranged from 6.3 to 7.1 for all the varieties, and in September the percentages lay between 1.7 and 2.5, which shows that during this period the ash increased much less than the dry matter.

When the mature Placentia walnuts were harvested from the 17-year-old trees on the irrigation plots being conducted near Irvine, California, representative samples of nuts were cracked for grading. In so doing kernels of varying degrees of shriveling were obtained, and these were classified according to the amount of shrinkage.

TABLE I

CHANGES IN FRESH WEIGHT, DRY WEIGHT, AND ASH CONTENT OF KERNELS  
OF THREE WALNUT VARIETIES

	PLACENTIA		PAYNE		SEEDLING	
	Date (1926)					
	6/13	9/21	6/13	9/17	6/12	9/23
Condition of kernel.....	Solid	Solid	Gel	Solid	Solid	Solid
Average* fresh weight per kernel.....	4.465 gm.	6.573 gm.	7.615 gm.	7.421 gm.	4.217 gm.	3.578 gm.
Average dry weight per kernel. . .	0.709	4.982	0.786	5.286	0.667	3.475
Average ash per kernel.....	0.047	0.091	0.530	0.104	0.045	0.085
Ash as percentage of fresh weight	1.05	1.38	0.70	1.40	1.07	2.38
Ash as percentage of dry matter.	6.63	1.84	6.73	1.97	6.69	2.45

\* Average of from 3 to 4 analyses with from 3 to 15 kernels per analysis.

Table II shows the results of analyses made upon normal mature kernels and upon those having various degrees of shriveling.

Five kernels of each lot were used for a single analysis, and the results in table II represent the average of four such closely agreeing analyses. With an increase in the amount of shriveling there was an increase in the percentage of ash in the dry matter. This gives an erroneous view of the situation, because the fresh weight, dry weight, and amount of ash per kernel all decrease with an increase in the shriveling. The decrease in the dry matter, however, is so large as to make the slowly decreasing amount of ash appear as an increase when the ash is calculated as a percentage of the dry matter. When the constituents of the ash are calculated as a percentage of the ash there appears to be an increase in the percentage of sodium,

potassium, calcium, sulphate, and inorganic phosphate with an increase in amount of shriveling.

Walnut kernels were obtained at different times of the year from the same trees in several locations and their ash composition determined. Ten kernels were used for a single analysis, and the values given in table III represent the average of four such closely agreeing

TABLE II  
EFFECT OF SHRIVELING UPON COMPOSITION OF MATURE WALNUT KERNELS

	NOT SHRIVELED	ONE-FOURTH SHRIVELED	HALF SHRIVELED	THREE- QUARTERS SHRIVELED	FULLY SHRIVELED
Ash as percentage of dry matter					
	2.24	2.81	3.22	3.77	4.84
Ash constituents (percentage of ash)					
Na.....	6.11	7.20	7.86	8.11	8.66
K.....	21.54	24.90	25.47	28.06	29.28
Ca.....	3.61	4.77	5.75	6.18	7.39
Mg.....	2.00	1.88	1.65	2.91	2.78
SO <sub>4</sub> .....	0.83	1.68	2.07	2.52	2.66
PO <sub>4</sub> .....	29.35	32.63	38.80	37.94	44.24
Grams per kernel					
Fresh weight.....	4.9642	3.5034	2.9947	2.3623	1.4207
Dry weight.....	4.8199	3.3836	2.8801	2.2585	1.3477
Ash.....	0.1242	0.0951	0.0916	0.0834	0.0640

analyses. The amount of dry matter increases at a more rapid rate than does the ash, and therefore the ash as a percentage of the dry matter shows a decrease as the season advances. The percentages of sodium, potassium, calcium, and sulphate in the ash show a decrease with an increase in the age of the kernels. The percentage of magnesium shows an increase, however, as does that of the inorganic phosphate in some varieties. The amount of each constituent of the ash per kernel in general shows an increase with the increased age of the kernel.

The effect of the degree of shriveling upon the total phosphorus content of mature kernels from the 17-year-old Placentia trees at Irvine is seen in table IV. The magnesium nitrate method was used



TABLE III  
COMPOSITION OF WALNUT KERNELS AT DIFFERENT STAGES OF DEVELOPMENT

	PLACENTIA (CITRUS EXPERIMENT STATION, BOX SPRINGS FIELD)			PAYNE (RIVERSIDE)		SEEDLING (RIVERSIDE)	
	7/13/26	9/21/26	10/20/26	7/13/26	9/17/26	7/12/26	9/23/26
Ash as percentage of dry matter..	6.72	1.82	2.35	6.73	1.97	6.92	2.51
Ash constituents (percentage of ash)							
Na.....	12.32	7.07	6.61	12.59	6.78	14.36	8.87
K.....	34.64	26.23	22.99	36.70	21.15	35.74	24.99
Ca.....	7.15	3.99	3.22	6.89	5.39	6.58	4.25
Mg.....	5.82	7.48	8.97	5.24	9.43	7.28	7.92
Cl.....	0.68	0.48					
SO <sub>4</sub> .....	3.77	0.98	1.02	2.59	1.15	1.75	1.11
PO <sub>4</sub> .....	45.88	51.54	60.38	45.94	44.37	55.65	56.17
Grams per kernel							
Fresh weight....	4.4652	6.573	7.906	7.6157	7.4216	4.7166	3.4023
Dry weight.....	0.7087	4.9828	5.034	0.7858	5.2862	0.6672	3.3009
Ash.....	0.0474	0.0909	0.1032	0.0529	0.1099	0.0445	0.0828
Na.....	0.0058	0.0064	0.0076	0.0066	0.0069	0.0064	0.0073
K.....	0.0164	0.0210	0.0250	0.0193	0.0219	0.0159	0.0206
Ca.....	0.0034	0.0036	0.0036	0.0036	0.0056	0.0029	0.0035
Mg.....	0.0027	0.0068	0.0093	0.0028	0.0098	0.0033	0.0066
Cl.....	0.0003	0.0005					
SO <sub>4</sub> .....	0.0018	0.0009	0.0011	0.0016	0.0012	0.0008	0.0009
PO <sub>4</sub> .....	0.0216	0.0468	0.0622	0.0239	0.0475	0.0247	0.0465

TABLE IV  
EFFECT OF DEGREE OF SHRIVELING ON TOTAL PHOSPHORUS  
CONTENT OF FIVE KERNELS

LOT	CONDITION OF KERNELS	DRY WEIGHT (GM.)	TOTAL PHOSPHORUS (GM.)
I	Normal.....	20.1422	0.0387
	One-fourth shriveled.....	16.6659	0.0373
	One-half shriveled.....	14.4557	0.0378
	Three-quarters shriveled.....	11.2879	0.0392
II	Normal.....	20.9800	0.0357
	One-fourth shriveled.....	11.6137	0.0372
	One-half shriveled.....	9.6418	0.0389
	Three-quarters shriveled.....	8.0922	0.0390
III	Fully shriveled.....	4.7416	0.0358
	Normal.....	18.6468	0.0360
	One-fourth shriveled.....	14.2174	0.0360
	One-half shriveled.....	13.2421	0.0396
	Three-quarters shriveled.....	9.9425	0.0382

to determine the total phosphorus in five kernels of a given lot. With an increased amount of shriveling, there was a reduction in the dry weight of the kernels. It will be seen, however, that there are no important differences in the total phosphorus content of the kernels, regardless of the degree of shriveling.

Although in table II a reduction in the amount of ash accompanies an increase in the degree of shriveling of the kernel, table IV makes it clear that there is no reduction in the amount of total phosphorus.

TABLE V  
TOTAL PHOSPHORUS CONTENT OF PLACENTIA WALNUT KERNELS AT  
DIFFERENT AGES DURING 1927

	DATE (1927)					
	JUNE 15	JUNE 30	AUGUST 2	AUGUST 11	SEPTEMBER 27	OCTOBER 5
	Kernel contents					
	Liquid	Gel	Solid	Solid	Solid	Solid
	Grams per 5 kernels					
Fresh weight.....	20.019	30.046	31.309	26.168	27.610	27.530
Total P.....	0.0125	0.0184	0.0408	0.0467	0.0678	0.0830
Total P calculated as PO <sub>4</sub> ..	0.0384	0.0563	0.1254	0.1430	0.2077	0.2543

The total phosphorus content of shriveled kernels is about the same as that of normal kernels, and hence the shriveling must take place after the kernel has received its full complement of total phosphorus, or else phosphorus absorption may still go on uninhibited by the shriveling process. It seems of interest, therefore, to make a study of the changes in the total phosphorus content of walnut kernels of different ages.

Placencia walnut trees were selected in the field at the Citrus Experiment Station, and at intervals as shown in table V samples of nuts were taken for analysis. On June 15 the kernel contents were still in the liquid condition. By careful manipulation the kernels were removed from inside the husks without losing any of their contents. Fig. 2 shows the appearance of the kernels at this time, with

some cut open to show the interior. By June 23 the tip and suture of the shells were becoming woody, and by June 30 the entire shell was woody. By July 7 the kernels were still liquid in the center, but firmly jelled along the inner surfaces of the kernel. At the time of the last sampling, the nuts were mature and ready to fall from the husks. A large number of nuts still in the husks were left after the determinations of August 2. These were left standing on the table, and on August 10, although some drying had occurred, the total phosphorus had not changed from that given for August 2. From table V it is seen that there is a progressive increase in the total phosphorus of walnut kernels as they increase in age. A comparison of tables IV and V shows a wide difference in total phosphorus between walnut kernels from the Riverside district and ones from the Irvine district.

### Summary

1. The shriveling of walnut kernels takes place after they have passed the liquid condition, have jelled, and are approaching the solid stage.
2. The fresh weight, dry weight, and amount of ash per kernel all decrease with an increase in the shriveling. The ash as a percentage of the dry matter increases with an increase in the amount of shriveling.
3. The amount of each inorganic constituent of the ash per kernel in general shows an increase with the increased age of the kernel.
4. The degree of shriveling has no effect on the total phosphorus content of mature walnut kernels obtained from trees growing on a given soil type.
5. There is a progressive increase in the total phosphorus of walnut kernels as they increase in age.
6. Normal mature walnut kernels obtained from trees growing on widely differing soil types show differences in their total phosphorus content.

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## BRIEFER ARTICLES

### TECHNICAL CONTRIBUTIONS

#### I. IMPROVED METHOD OF SOFTENING HARD TISSUES

In various contributions, methods have been described for studying microscopically the organization of hard vegetable materials such as coal, carbonized fossil plants, dense tropical woods, etc. In the case of the more refractory of these substances, the prolonged treatment necessary for adequate softening has proved a disadvantage; and the tissues themselves in some instances suffer modification from too lengthy treatment. As a consequence, experiments have been carried on during the past two years for the purpose of shortening and at the same time improving the technical manipulation of hard vegetable objects. These experiments have now reached a successful conclusion, and in view of the considerable interest which they have aroused they are published herewith.

The fundamental condition in the newer technique of softening vegetable tissues is the use of the somewhat high temperature employed in the vulcanization of rubber. An ordinary dental vulcanizer has proved to be very satisfactory, especially as the small ones suitable for this purpose are not very expensive. Ordinarily they are equipped with a thermostat which controls the temperature at 320° F. At first the material was raised to the vulcanizing temperature in water, as boiling water is ordinarily employed in preparing woods for sectioning. This procedure answered fairly well in many instances, but unless care was taken there often resulted an extreme degree of hydration which made the tissues friable and difficult to section. It was considered probable that the use of alcohol of various grades would overcome this difficulty. In 95 per cent alcohol very little hydration or disintegration occurs and the period for softening is somewhat prolonged. For example, a piece of white or red oak will require three or four hours or even more in the thermostat for proper softening. If 70 or 50 per cent alcohol is used the length of time required is shorter, but the danger of deterioration is greater. If pure water is used with oak half an hour is quite sufficient, but the result is not nearly so good as that obtained by exposing the same material in 95 per cent alcohol for four hours. After treatment in the vulcanizer a very brief action of hydrofluoric acid is sufficient. Even in the case of the

hardest materials, two or three days' exposure to hydrofluoric acid of one-half or one-third the commercial strength is all that is necessary.

One great advantage of the new method is that it is possible to treat with it tissues which are partly hard and partly soft; for example, the branches of oak and hickory are more or less difficult to soften satisfactorily by the older method. When vulcanized in 95 per cent alcohol they are quickly mastered, and are much more homogeneous than they are as the result of prolonged treatment in hydrofluoric acid alone. The time of exposure of a three- or four-year old branch of oak naturally is much shorter than for a piece of dry oak wood. An hour generally suffices, in contrast to the four or five hours required for the wood.

The technique of preparing the material for vulcanization in alcohol is of considerable importance, although easily and inexpensively practiced. Pieces of "iron-size" brass pipe 0.75-1 inch in diameter are cut to fit in the vulcanizer. These short lengths of pipe are threaded at each end for a brass cap of appropriate size. One end is capped and the cap is made vapor-tight by having lead solder "sweated" into the thread. The cap on the other end has inserted within it a plate of lead against which the smooth end of the pipe fits. The lead gasket, however, does not serve alone to prevent the loss of alcohol at high temperatures. It has been found desirable to insert a disk of cork, fiber, or common cardboard between the lead gasket and the end of the pipe, thus securing a perfectly tight joint even with strong alcohol. The brass pipe is fastened in a pipe-vise, and after the material has been introduced in alcohol the cap is firmly screwed on with a Stillson wrench. Obviously a number of samples can be treated in the same or different tubes in one operation, with a corresponding economy of time. After vulcanizing the material the vulcanizer is allowed to cool slowly, or if haste is necessary the steam is allowed to escape and the hot brass tubes are cooled under a faucet. When the material is removed from the tube it needs only a comparatively short sojourn in hydrofluoric acid. Admirable results have been obtained without imbedding in the case of such refractory materials as peach stones, coconut shells, and the shells of a great number of nuts. Equally good results have been secured in the case of such woods as oak and *lignum vitae*. After these various hard objects have been vulcanized they are treated for a few days in a mixture of one to two parts of water to one of strong hydrofluoric acid. After being well washed in water they are run up in alcohol and finally transferred to a mixture of equal parts of alcohol and glycerin, as in the earlier methods.

There is no comparison between the results to be obtained in this way

and those secured by the older technique. For example, it is possible to cut continuously transverse sections of coconut shells and hard woods only 2 or 3  $\mu$  thick. The wealth of detail shown by these sections which are entirely without scratch or dragging on the part of the knife is almost beyond belief. Every feature of pitting stands out with diagrammatic clearness, and the most difficult details can readily be studied with the use of an oil immersion. The treatment here described yields such satisfactory results that it seems to mark an important step forward in the difficult field of the study of hard tissues of plants.

## II. MASS METHOD OF IMBEDDING

In the practice of microtomy, occasions often arise where it is a great advantage to be able to cut a large number of small objects at the same time, such as the carpels of the rose, or the small flowers of the sedges, grasses, or Compositae. Obviously, if the carpels of the rose are sectioned as they lie in the young fruit, or the flowers of the groups mentioned are cut on the microtome in heads, comparatively few of them will reveal the median section which is of such value in arriving at conclusions in any given case. This situation presents itself in its most difficult form in the study of the reduction division, particularly in ovules and very small anthers. In the case of ovules there are comparatively few mother cells undergoing division in a given quantity of material, and the same statement applies to the small anthers of the minute flowers of many groups of plants. In such instances it is obviously advantageous to cut great quantities of material at once and in the desired plane of section. The mass method here described has been developed to meet these difficulties.

The material, however preserved (the method varying of course in different cases), is run up into strong alcohol, and then transferred to a mixture of equal parts of alcohol and glycerin in which it should stay at least overnight. When the glycerin-alcohol has passed into the material, the objects are removed from it and handled under the low power of the dissecting microscope. Let us suppose that the ovary of a rose is to be studied in order to discover the behavior of the chromosomes in the heterotypic divisions of the embryo sac mother cell. Before transferring to glycerin-alcohol the top of the flower has been cut off below the level of the stigmas, and a similar section at the time of preservation has laid open the base of the receptacle. The young flower is placed on the stage of the dissecting microscope, and with needles or fine forceps has the base of the calyx surrounding the carpels removed. The bunch of carpels is then transferred to a piece of heavy paraffin paper in a small drop of

equal parts glycerin and alcohol. The carpels are separated from one another by needles or forceps and laid out in a row on the piece of paper. Obviously, several flowers may be advantageously manipulated at once. After the carpels are laid out properly, the strip of paper is put in a warm place to encourage evaporation of the alcohol.

Previously a supply of glycerin jelly is prepared by using pulverized gelatin, purchasable at any grocery. The gelatin is put in warm water in the proportion of one ounce of gelatin to six ounces of water. After the gelatin is thoroughly dissolved, which happens very quickly if it is pulverized, an equal volume of glycerin is added. The jelly so produced resembles closely that ordinarily employed for mounting certain microscopic objects. It is, however, unnecessary to filter or clarify it for the present purpose. Further, unless a very large stock is made up it is unnecessary to use an antiseptic, as the glycerin serves this purpose sufficiently.

Cardboard, which need not be of a very high grade, is cut up into strips of a convenient width, depending on the particular material used. Eighteen mm. is a generally useful dimension, but sometimes broader strips are required. Some of the glycerin jelly is heated to the melting point and spread with a glass rod or the limbs of a forceps evenly over a small strip of cardboard. The objects which have been arranged on paraffin paper in any desired order, having in the meantime lost their excess of alcohol, are inverted and applied to the melted glycerin jelly, the paraffin paper naturally being uppermost in this operation. After the paraffin paper has been placed over the glycerin jelly, an ordinary microscope slide is laid on the top of the paper and on the slide is placed a small weight which flattens out all the rose carpels parallel to the surface of the cardboard. When the jelly has set (from half an hour to two hours is usually long enough), the strips of cardboard with their adhering paraffin paper are put in 90 per cent alcohol, which brings about the hardening of the jelly. Sometime afterward, it may be next morning, the cardboard strips are placed in a watch glass of alcohol. The paraffin paper has now become translucent, and it is possible at this stage to proceed to the further preparation of the material. This is effected by pricking with a fine (no. 12) needle inserted in a cork. The pricking should be somewhat uniform, but care should be taken to avoid perforating parts likely to be injured by that operation. The paraffin paper may now be pulled off. This method has been arranged especially for nitrocellulose infiltration. It is possible by means of a comparatively few sections through the median plane of a large number of carpels to secure a large amount of important information.

The gelatin used in this method, if employed too abundantly, constitutes a deeply staining matrix surrounding the objects. By using a thin layer of gelatin it is possible to bring about the adhesion of the objects without any of these disadvantages.

The objects prepared by this method stain very well and supply most useful preparations. By the employment of this method it is possible to use material for class purposes which without this procedure could not possibly be so utilized. Doubtless this method could also be employed with paraffin, but it has proved its great utility in connection with the imbedding of material in nitrocellulose.

### III. SLIDING MICROTOME FOR CUTTING HARD TISSUES

(WITH ONE FIGURE)

An outstanding necessity for American biologists has been a microtome on the sliding principle, which would combine extreme accuracy with the possibility of securing thin sections, particularly of hard tissues. A microtome, fulfilling these requirements to a very large degree, has been devised by Professor R. B. THOMSON of the University of Toronto, and has been so suitable for its purpose that it has attained a wide although somewhat limited sale. The price of this microtome, in the neighborhood of \$500.00 in Toronto where it was manufactured, has put it beyond the reach of most workers in other countries, particularly since the war and the consequent prevalence of high tariffs.

A microtome of this type has been so much a desideratum for American workers in recent years, particularly as a result of the increased interests in anatomy and the greater use of nitrocellulose as an imbedding medium for all purposes, that the writer has considered the construction of a modification of this microtome. The cost of the Thomson instrument is due very largely to the somewhat expensive appliances which have been introduced into its structure, chief of which is the apparatus for raising, lowering, and tilting the knife. This feature of the Thomson instrument has been admirably designed, but is too elaborate and expensive for general use. The writer has taken up the subject of modification of the Thomson microtome with several firms of instrument makers, with the result that the Bausch and Lomb Optical Company three years ago undertook to manufacture and put on the market a modified Thomson microtome.

The new microtome (fig. 1) is provided with a micrometer screw of the clicking type. This differs from the one supplied with the first instrument and has certain practical advantages over it. The micrometer



screw on the original instrument is modeled after a type now commonly used in which the movement of the screw is controlled by a lever and ratchet. The lever in this type of appliance, if used with the vigor necessary to overcome the inertia of the rather heavy block which supports the object, stubs against the rod which controls the thickness of the section. This is injurious to the micrometer apparatus, and there is great difficulty in securing the particularly thin sections which are desirable, not only in difficult investigations, but also for general use in the classroom. The older type of micrometer screw, in which a knife-edge engages in a transverse groove of a disk attached to the screw, is of greater practical advantage, since it permits much smarter and more vigorous action, which is necessary to overcome the inertia of the object-holder, particularly in the case of very thin sections. Further, the operation does not endanger any part of the screw itself. The only fault which can develop is over-shooting the required thickness, a defect which is quickly remedied as the microtome becomes easier running by use and the operator has acquired skill and experience.

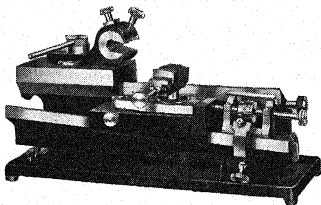


FIG. 1

The instrument is constructed of nicked steel, and in it the three point bearings are inverted from the position ordinarily found in sliding microtomes of European manufacture. This inversion makes for greater rigidity and solidity in operation, and appears to constitute a decided feature of advantage in the Thomson instrument as well as in its modified form here described. The object-holder has a very efficient universal joint which is so large that great rigidity results. The object clamp is a very practical type, and is provided with three different insets for cutting round objects of various sizes. This feature is highly advantageous. The object-holder is pushed up an inclined plane which has a gradient of one in twenty, as in the well known Jung-Thoma microtome, which has supplied the needs of American workers for many years. The knife-holder, as is usually the case, runs on horizontal ways. The knife is held by a tilting knife-holder which can be oriented at any angle in the vertical and horizontal planes. The raising of the knife for long objects is attained

in the new instrument by the efficient and inexpensive device of plane-parallel plates. A number of these are supplied, and permit a considerable range of height.

The new instrument has been thoroughly tested in the writer's laboratory. It is accordingly probable that it will not develop any important defects when used for the purposes for which it was designed, namely, the sectioning of hard tissues and of objects imbedded in nitrocellulose. In use it has proved equally advantageous for animal and vegetable material, and it is confidently recommended to American biologists as an instrument which will give the maximum of performance at the lowest consistent price.

#### IV. IMPROVEMENTS IN USE OF NITROCELLULOSE FOR IMBEDDING

(WITH ONE FIGURE)

Imbedding in nitrocellulose has become more and more important in the technique of both plants and animals in recent years. It is now possible to cut sections as thin as those which may be prepared by the paraffin method in the most favorable cases. Not only is nitrocellulose now applicable to the more delicate tissues of plants, but it is still the method par excellence for hard tissues and for structures combining thick- and thin-walled cells. Under certain conditions it may produce shrinkage, in spite of its well deserved reputation for causing less disturbance in this respect than does paraffin. The general technique which has proved of value in the writer's laboratory is adequately described by CHAMBERLAIN,<sup>1</sup> or at greater length by the writer.<sup>2</sup>

It has been found desirable, in connection with cytological investigations on the more delicate parts of plants, and on more delicate organisms than have previously occupied attention, to develop improvements in the technique of imbedding in nitrocellulose. These improvements offer advantages in connection with staining, and furnish distinctly clearer pictures than can be obtained with the paraffin method.

It will be convenient to consider first the preparation of very delicate structures for the purpose of imbedding. Perhaps the most delicate and difficult objects in this connection are the green and brown algae, as, for example, *Spirogyra* and *Fucus*. The classic and apparently the most advantageous preservative in these cases is chromic acid, or rather chrom-acetic varying in strength from one to a fraction of one per cent, depend-

<sup>1</sup> CHAMBERLAIN, C. J., *Methods in plant histology*. 4th ed. 1924 (pp. 127-129).

<sup>2</sup> JEFFREY, E. C., *Anatomy of woody plants*. 1917 (pp. 449-451).

ing on circumstances. The material of *Spirogyra* is dropped without preparation into the preservative. In the case of *Fucus* it is desirable to cut off the ends of the so-called receptacles, so that the chromic acid may penetrate readily. In both instances it is advisable to use an air pump (to be subsequently described) in the field, for the purpose of removing the gases and promoting penetration of the preservative. After remaining for one or two days in the preserving fluid, the material is washed with water to remove all excess of chromic acid. If the material is not to be used at once, it may be kept indefinitely in water to which 5 per cent phenol has been added. In case of the brown seaweeds it is desirable to demineralize before imbedding, as there is some mineralization of the thallus near the surface which interferes with the preparation of thin sections for cytological study. The removal of mineral matter is effected by weak hydrofluoric acid (not stronger than 5-10 per cent). Washing removes the acid, and the material is now exposed to the action of 10-15 per cent glycerin in water. The glycerin is concentrated by the slow evaporation of the water, which is facilitated by a very slight degree of warmth. This procedure is commonly adopted for the purpose of making total preparations of algae, but is here utilized for making sections free from shrinkage in the case of even the most delicate objects.

After the glycerin has become concentrated by slow evaporation of the water, the material is transferred to strong alcohol (90-95 per cent). If the objects are massive they do not require any special handling, but in the case of *Spirogyra* and other minute or filamentous algae it is necessary to attach a mass of the material to a piece of cardboard by means of the mass method previously described. To effect this, the material of *Spirogyra*, *Chara*, or *Nitella*, etc., is changed from strong alcohol to equal parts of alcohol and glycerin. From this it is transferred to blotting or filter paper, to allow some of the alcohol to evaporate. A mass is then lifted to a piece of cardboard coated with a thin layer of glycerin jelly, and then gently weighted down after having been covered with a piece of heavy paraffin paper and a glass microscopic slide. This is preliminary to imbedding. In the case of *Fucus*, *Laminaria*, etc., it is often desirable to prick the material to some extent with a fine needle, to permit subsequent penetration of nitrocellulose. Whether the algae are small or large (the same treatment applies to aquatic and other fungi), they are now transferred to strong alcohol and the air removed with a powerful air pump. They are then dehydrated in the usual manner by successive changes of absolute alcohol, three in number. The material now passes in 12-hour periods through nitrocellulose in 2, 4, 6, 8, and 10 per cent

solutions, which are heated in a carefully wired bottle in the bath. The temperature of the bath may be  $50^{\circ}$ – $65^{\circ}$  C. and even hotter. The hot nitrocellulose penetrates much more thoroughly and much more quickly than when used cold, and very thin sections can readily be secured as a consequence. Instead of passing the material through successive higher grades of nitrocellulose, as is the case with less delicate material, the thickening process starts with the 10 per cent solution.

Fig. 2 will make clear how this is brought about. A piece of copper or zinc is cut into strips about an inch wide with a pair of tinsmith's "snips." These strips are cut crosswise at intervals of two inches and then one end is sharpened to an arrow-shaped point. The strip of metal is then folded lengthwise through the middle over a piece of hard wood to

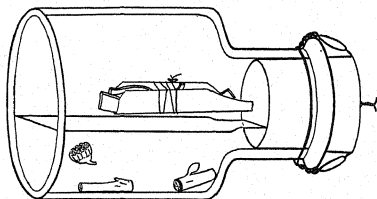


FIG. 2

an angle of  $90^{\circ}$ . One or two turns of soft brass wire are wrapped round the resulting trough somewhere near the middle. Under this wire are pushed strips of parlodion or photoxylin until wedging occurs. The material in 10 per cent nitrocellulose is now ready for the thickening process. This is effected by driving the pointed end of the metal trough, with the concavity upward, into the inner end of the cork. This should be done so that the lower side of the trough does not more than touch the surface of the nitrocellulose when the bottle is laid in a horizontal position. The bottle is then securely wired and put into the warm bath. At the end of 24 hours it is cooled off and the cork withdrawn. A second trough filled with nitrocellulose is substituted for the first, and after a sojourn of 24 hours the process is repeated.

This method has the advantage of bringing about a very gradual concentration of the nitrocellulose which is superior to the ordinary evaporation method. In the method by evaporation the ether passes off from the solution more readily than the alcohol, and a more or less cartilaginous

mass results which neither supports nor penetrates the object thoroughly. By the method described, alcohol and ether are equally absorbed by the suspended nitrocellulose. There is consequently no precipitation due to excessive evaporation of one of the solvents; and shrinkage, such as results from the direct placing of chips of nitrocellulose in the dissolved medium, is also avoided. It is obvious that it is possible by this method to make the thickening extremely gradual and also progressive. This makes it possible to imbed the most delicate algae and fungi without shrinkage. After hardening the nitrocellulose in the usual manner, sections may be cut readily  $5\mu$  or even thinner. This procedure has proved of great value in manipulating saprophytes, fungi, etc., which on account of their poorly developed air spaces are apt to shrink with the ordinary method. For example, the ovules, ovaries, and fungus covered roots of our two species of *Monotropa* can readily be prepared for sectioning in this fashion. The same statement holds true for the delicate mycorrhizas of the orchids, which constitute an exceedingly difficult object with the usual technique.

It may appear that the process of imbedding here described is too elaborate for ordinary use. The main labor, however, is in getting the material into 10 per cent nitrocellulose. After that stage has been reached the thickening of the imbedding medium is practically automatic. The sections which result are very clear, and serve admirably for photomicrographic purposes. They may be stained in almost any desired manner, provided the staining is effected slowly by weak solutions of the dyes. Haidenhain's haematoxylin works equally well with the various anilines.

## V. AIR PUMP FOR THE FIELD

(WITH ONE FIGURE)

It has been realized more and more in recent years that the exhaustion of air from vegetable tissues is a prime necessity from the standpoint of good preservation. When preservation is carried on in the laboratory the electric air pump, which has become a necessary adjunct of modern biological laboratories, is available for this purpose. Since, however, a very large part and a vitally important part of the preservation of material must be effected in the field, a suitable air pump for use under field conditions is highly desirable. The automobile pump is the inexpensive and entirely satisfactory basis for such an instrument. The hand pump for the automobile is constructed to force air into the inner tube of a tire. It is possible with a few slight changes to make it function efficiently as a producer of negative pressure. The left-hand items of fig. 3 show how this is effected. All that is necessary is to reverse the leather

plunger, and in so doing make sure that it is held firmly in place in its new position. In some pumps it will be necessary to put a washer on the plunger rod, just above the leather piston, as otherwise the piston will not fit tightly in place. When the nut which holds the piston is screwed up it is well to rivet the end of the rod, so that the nut will not come off and thus cause annoyance in the field. Care should be taken also to have

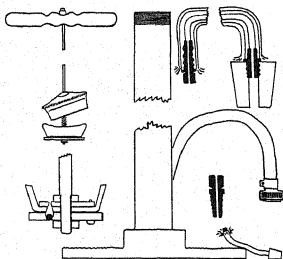


FIG. 3

a spiral spring between the top of the plunger and the cap of the pump, to take up the shock of the upward stroke and thus prevent the cap from being torn off. Usually there is a ball valve at the base of the hose attached to the pump. By unscrewing the nipple to which the hose is attached the ball can be readily extracted. This is a necessary operation because the ball valve responds to positive pressure and gets in the way of negative action.

The hose from the pump has attached to it the valve of the inner tube of an automobile tire. This is wired into a short length of rubber hose which at the other end has another valve from a disused inner tube. The rubber plunger or valve inside is removed from one or both of the valves. In certain instances it is an advantage to keep it in the valve farthest away from the attachment. This valve is inserted into a rubber stopper which fits the bottles used for preserving. The details of these structures are represented on the right of fig. 3.

To use the pump, the rubber stopper is inserted into the bottle containing the preserving solution and the material to be preserved. The plunger is drawn smartly upward but the stroke stops short of the air vent in the side of the cylinder just below the cap. In this position a high vacuum is instantly produced, which causes the air to gush out of the material. As soon as the flow of air has diminished, the plunger is drawn to the top and the vent allows the air pressure to pass off. The plunger is then forced down slowly and drawn back with another sharp stroke. Four or five strokes are sufficient to cause most objects to saturate and sink in the preserving fluid. When an automobile pump is used, a valve inside is not necessary in the tube inserted in the rubber stopper,

as the space in the cylinder of the pump is relatively large compared with the bottle to be exhausted. If the automobile pump is replaced by a bicycle pump, as has been suggested by Professor R. H. WETMORE, a valve is essential on account of the short stroke and the narrow diameter of the pump. Practically the valve has been found unsatisfactory, however, on account of the frequent attention which it requires, and its very short life resulting from the corrosive vapors to which it is exposed. The writer for that reason does not use a valve at all.

The piston of the pump is most suitable for the purpose, if provided with a ball valve as shown on the left of the illustration. The advantage of the ball valve is that the piston in its downward stroke allows air to pass through readily, and does not force the cork out of the bottle which is being exhausted. Care should be taken to clean and grease the leather of the piston frequently, on account of the character of the preservative fluids used for the fixation of the tissues.

This pump has been found useful, not only for vegetable tissues but for animals as well, as in all cases it favors the rapid penetration of the preserving fluid. A further advantage is the fact that a smaller amount of preservative can be safely used and larger pieces of material than where no pump is used. This appliance has now been in use in the writer's laboratory for nearly a decade, and the present account is supplied as an easy way of answering numerous inquiries on this subject.—E. C. JEFFREY, *Harvard University*.

# CURRENT LITERATURE

## BOOK REVIEWS

### Morphology of fungi

During the past 20 years, botanical literature has been accumulating at such a rate that it has become increasingly difficult for investigators, especially those of the younger generation, to make sure that they are not overlooking work already done. *Botanical Abstracts*, and now *Biological Abstracts*, help to minimize the danger. Fortunately, it has been recognized that the danger exists, and useful books are appearing which put the student into contact with the most important literature in a given field and lessen the drudgery of reference library work. Some notable examples of this type of book are *Cytologie der Blütenpflanzen*, by SCHÜRHOFF; *Anatomie der Angiospermen Samen*, by NETOLITZKY; and in our own country, the works on cytology by WILSON and SHARP. In all these books a clear presentation of the subject, with very extensive bibliographies, greatly facilitate research.

A recent work of this character, which will be of great value to all who are studying fungi, is DODGE'S<sup>1</sup> translation of GAÜMANN'S *Vergleichende Morphologie der Pilze*.

The book is more than a translation, for the new literature which has appeared since 1925 has been incorporated, and in some places the recent results have necessitated a rewriting of the group concerned. Some of the more important of these changes are seen in the treatment of the Basidiobolae, the Elaphomycetaceae, the stromatic Sphaeriales, the Laboulbeniales, the Thelephoraceae, the Hydnaceae, the Gasteromycetes, and the Uredinales. An extensive rearrangement of orders was made with GAÜMANN'S approval. Unfortunately, Dr. GAÜMANN'S illness made it impossible for him to collaborate in the work.

The chapter headings indicate the arrangement of groups and suggest something of the mode of treatment. They include a discussion of the Thallus, Reproductive organs, Sexual organs and sexuality, Archimycetes, Phycomycetes, Chytridiales, Oomycetes, Zygomycetes, Ascomycetes, Hemiascomycetes, Taphrinales, Euascomycetes, Perisporiales, Myriangiales, Hypocreales, Sphaeriales, Dothideales, Hysteriales, Hemisphaeriales, Phacidiales, Pezizales, Tuberales, Laboulbeniales, Basidiomycetes, Polyporales, Agaricales, Gasteromycetes, Tremellales, Cantharellales, Dacryomycetales, Auriculariales, Uredinales, Ustilaginales, and Fungi Imperfecti.

<sup>1</sup> DODGE, C. W., Comparative morphology of fungi. 8vo. pp. xix+701. figs. 406 and 43 diagrams. New York: McGraw-Hill Book Co. 1928.



The book is written from the standpoint of morphology, with cytological and phylogenetic features everywhere in evidence, the opening paragraphs introducing the  $x$  and  $2x$  features of alternation of generations. The statement that when the  $x$  and  $2x$  phases follow each other as two morphologically different generations we have alternation of generations is true; but the reviewer would contend that there is alternation of generations wherever there is sexuality, whether the two generations look alike or different. That the  $x$  generation is called the gametophyte and the  $2x$  generation the sporophyte is true, and it happens to be correct for plants above the Thallophytes, but is unfortunate for the algae and fungi, where the  $x$  generation so often produces both gametes and spores. We must also take exception to the statement that the  $2x$  phase is "reduced to a zygote." More probably it has not been reduced; but rather the reduction division, bringing back the  $x$  phase, has simply taken place early, before a more extensive  $2x$  phase could be built up. This short  $2x$  condition should be expected in the evolution of alternation of generations at the time when reproduction by gametes began to emerge from the zoospore condition.

The fact, however, that alternation of generations is recognized and emphasized, gives the book a freshness not found in works where the morphology is limited to a study of disconnected life histories, with the control of diseases as the only object.

The treatment of the Laboulbeniales and Uredinales is particularly full, and is rewritten and revised rather than translated. Similar but less extensive changes are found throughout the book.

The illustrations are numerous and well chosen. As should be expected in a work of this scope, very few are original. Some of the figures are taken from papers which appeared after GAÜMANN's book was published.

To mention individual details would require more space than can be given to a review. The whole treatment is from the standpoint of comparative morphology, with cytology and phylogeny prominent. The clear presentation, with more than a thousand titles in the bibliography, and an effective index of 30 pages, will greatly facilitate research by putting students into contact with the literature, and systematizing the reference library work necessary in any investigation. Morphologists, pathologists, and mycologists will find this book very useful, even if they already have the German edition.—C. J. CHAMBERLAIN.

#### Life of plants

An attractive book on the life of plants has appeared by Sir FREDERICK KEEBLE<sup>2</sup> of the University of Oxford. It is an exceptionally entertaining and instructive work, intended for the lay reader and for those who are just beginning the serious study of plant life. Likening the development of science to the growth of a tree, KEEBLE attempts to show that science is much more than a mere body of doctrine, that it is, indeed, an illumination of life. Admitting the

<sup>2</sup> KEEBLE, F., *Life of plants*. 8vo. pp. xii+256. New York: Oxford University Press, American Branch. 1926. \$1.75.

difficulties of presenting science in this rôle, he modestly expresses the opinion that he has failed to measure up to the task. Nevertheless, the reader will find the work an unusually illuminating introduction to the world of plant life. Botany would gain much in appreciation of its service to humanity if the knowledge to be obtained from this book were to become the possession of people generally.

This interpretation of plant life is presented in nine chapters, the first of which deals with the cosmic significance of plants, the part they play in nature, and the origin of the land flora. In the second chapter the chlorophyll-bearing and non-chlorophyllous plants are contrasted, and wheat is described as an example of useful seed plants. The third and fourth chapters consider the food-manufacturing activities of the higher plants, including the utilization of nitrogen, and the way in which radiant energy of sunlight becomes the potential energy of the foods stored in the plant body. The fifth and sixth chapters present the problems of mobilization of food materials, diffusion, osmotic transfer, the conducting system, enzymes concerned in mobilization, and the final use of foods for constructive material and vital energy.

The final chapters consider the environment of land plants, variation and heredity, and the plant commonwealth. The last chapter centers attention upon one of the most difficult problems of life, correlation of life activities, the integration and coordination of the processes upon which orderly development and the fulfilment of the purposes of life depend. This he conceives as dependent mainly upon chemical messengers, hormones, set free by the stimulative action of environmental conditions on the sensitive protoplasmic material of the cells.

The story is told in pleasing manner, and the book should prove of value as collateral reading for students who are gaining their first serious knowledge of plant life and plant processes. The reviewer considers that KEEBLE has given us an excellent elementary illumination of the life of plants as active living beings.—C. A. SHULL.

#### Principles of plant physiology

The greatest need with reference to textbooks in botany at present is for a good sound text in plant physiology. Few attempts have been made in recent years to meet this need, and those have been mostly abortive efforts. The latest work along this line is a text by RABER.<sup>1</sup> The book contains thirty-one chapters, the titles of which will not be given. The six sections, however, are: Introduction; Nutrition; Growth and movement; Reproduction and heredity; Death; and Vitalism and mechanism.

The introduction classifies the sciences, and considers the cell as the unit of living structures. The colloidal state is considered in connection with the properties of protoplasm. The section on nutrition is presented in twenty-three chapters, and falls into three divisions: Constructive metabolism; Absorption and movement of materials; Destructive metabolism.

<sup>1</sup> RABER, O., *Principles of plant physiology*. 8vo. pp. xvi+377. Macmillan. 1928.

Certain features of the arrangement seem illogical to the reviewer. For instance, the problems of absorption and movement of materials, gases, liquids, salts, etc., and the problems of transpiration and ascent of sap are more easily understood than the problems of photosynthesis, chemosynthesis, and nitrogen metabolism. They should precede the latter subjects for an easier approach to the problems of plant life. In destructive metabolism, anaerobic respiration is considered after aerobic, even though in actual life probably all respiration has an anaerobic start. The logical arrangement is to treat the subject of respiration as a whole, and start at the beginning of the process.

In some chapters the text somewhat overshoots the mark for an elementary presentation, but in the main it could be mastered by beginners. The chief criticism to be made is that there are many errors running through the text which students will not be able to detect because of lack of experience and judgment. It is not possible to go into details with very many of these errors, but a few cases will be cited. On page 50 the author indicates that BALLY's earlier work on photosynthesis rests upon careful experimental evidence. The facts are that BALLY's theory is highly hypothetical, not based upon experimental results, and not in agreement with WILLSTÄTTER's published work. Again, on page 107 the author indicates that the salts in plants are maintained in constant ratios, similar to the condition of the salts in the blood of animals, and that the ratios are related to the Cambrian Sea out of which life is supposed to have arisen. This idea certainly does not receive any support from the known facts of salt distribution in the sap of plants.

On page 114 it is stated that d-xylose is optically inactive, although ARMSTRONG and others give the rotation for the  $\alpha$ -form as  $+92^\circ$ ; and on page 112 an erroneous interpretation of the prefixes d- and l- for the optically active sugars is given. Take fructose, for example, levorotatory whether in the  $\alpha$  or  $\beta$  form, and levorotatory at equilibrium of the two forms in mixture, yet it is a d-sugar. On page 153 the impression is given that emulsin is a single enzyme, but, like diastase and other similar preparations, it represents a group of enzymes of which amygdalase and prunase are the principal catalysts. On the following page, amygdalin is made "the essential material in oil of bitter almonds"; but oil of bitter almonds usually signifies benzaldehyde, which is a constituent of amygdalin.

On page 166 it is not made clear that litmus has no relation to the anthocyanin pigments, along with which it is mentioned. The student would get the idea that litmus belongs among the anthocyanins. On page 279 the "grand period of growth" is interpreted as the central part of the growth period, when growth is most rapid, whereas the term is used for the total growth period, from the very beginning of enlargement until growth ceases. On page 293 ethylene is credited with ripening lemons, although its action is merely to hasten chlorophyll decomposition in lemons which are internally ripe. Many other instances might be cited, but these cases are sufficient to indicate the kind of errors which occur.

While the number of these slips is disappointing, one finds that many of the

chapters are otherwise well written; some are fair in quality, and about half a dozen are rather poor. It is greatly to RABER's credit that he has attempted to write a text for the use of plant physiologists, a task which has been slighted by those well fitted by training, experience, and research to perform. A good text cannot appear unless those capable of writing one are willing to sacrifice time and other forms of scientific activity in order to produce it. Such a text should come if possible from a master teacher who also possesses ability as an investigator, and who is capable of exercising discriminating judgment as to the material which should be included in such a text.—C. A. SHULL.

#### First course in botany

Another elementary text has recently been prepared by POOL and EVANS.<sup>4</sup> The first chapters deal with customary topics: cells, roots, leaves, stems, the flower, and the seed. The emphasis is placed upon the work of these parts of the plant, with only sufficient discussion of structure to afford a basis of understanding. Then follows a chapter on the plant and its environment, with another on weeds as application of the adaptation of the organism to its environment. Chapters x to xv, 133 pages, deal with classification and with type plants. The closing chapters are on forestry, plant breeding, and the diseases of plants.

The language of the book is simple and the authors have been conservative in the introduction of scientific terminology. At the close of each chapter there are a summary and a series of questions and problems. These latter are intended to test the pupil's ability to apply the principles treated, rather than merely to recall the information acquired.

The book may be commended as an excellent text for secondary school botany. It has been written with the editorial cooperation of Dr. OTIS W. CALDWELL, and is evidently intended to bring up to date the earlier books of which he was an author.—E. R. DOWNING.

#### Useful plants

Aside from the usual products of farm and garden, it is surprising how few useful plants are known to the general public. To furnish means of a more extensive acquaintance, CLUTE<sup>5</sup> has issued a little book, written in non-technical language, describing many plants used as food, condiments, beverages, drugs, dyes, textiles, construction, and decoration. The book should be of special interest to the layman, and even the professional botanist may learn something from its pages.

In general the common names of the plants are used in the text, but there is also a list giving the botanical names and the family to which the plant belongs. The book should serve to emphasize the economic importance of all branches of plant science.—G. D. FULLER.

<sup>4</sup> POOL, R. J., and EVANS, A. W., *First course in botany*. 8vo. pp. ix+414. figs. 219. Ginn & Co. 1928.

<sup>5</sup> CLUTE, W. N., *The useful plants of the world*. 8vo. pp. 86. Joliet, Illinois: Publ. by W. N. Clute & Co. 1928.

## NOTES FOR STUDENTS

**Taxonomic notes.**—TRELEASE<sup>6</sup> has published a new genus (*Trianaepiper*) of Piperaceae, which includes 4 species segregated from *Piper*. The author states that these are "the only other species with axillary spikes which remain in the genus *Piper*." In range they are confined to the Colombian Mountains.

BROWN<sup>7</sup> has described a new genus (*Cerochlamys*) of Mesembryanthemeae from South Africa. The name refers to the waxy film that covers the leaves.

In continuing his account of African plants, GOSSWEILER<sup>8</sup> has described a new genus (*Dalbergiella*) of Leguminosae, a shrubby climber closely related to *Dalbergia*.

PITTIER<sup>9</sup> in continuation of his investigation of the Bignoniaceae of Venezuela, has described 9 new species of *Arrabidaea*, almost doubling the number of species known in that country.

FERNALD<sup>10</sup> has described a new genus (*Geocaulon*) of Santalaceae, to include what has been known as *Comandra livida*. It creeps in moss or damp humus, and occurs from Labrador to Alaska and southward to northern New England and British Columbia.

ROBINSON<sup>11</sup> in recording material preliminary to his publication of the Eupatorieae, has described as new genera *Mexianthus* (Mexico) and *Trychinolepis* (Peru); and also 4 new species of *Stevia* from Mexico and 12 new species of *Eupatorium* and 6 new species of *Mikania* from various South American countries.

JOHNSON<sup>12</sup> in continuation of his investigation of the Boraginaceae, has published an account of the South American species of *Heliotropium*. The genus contains 73 species, 13 of which are described as new. He has also published<sup>13</sup> 19 new species from various South American countries in 17 genera, one of which, belonging to the Iridaceae, is new (*Mastigostyla*).

<sup>6</sup> TRELEASE, W., *Trianaepiper*, a new genus of Piperaceae. Proc. Amer. Phil. Soc. 67:47-50. 1928.

<sup>7</sup> BROWN, N. E., *Cerochlamys*, a new genus of Mesembryanthemeae. Jour. Botany 66:171, 172. 1928.

<sup>8</sup> GOSSWEILER, J., Plants from Angola and Portuguese Congo. Jour. Botany Suppl 66:121-136. 1928.

<sup>9</sup> PITTIER, H., Studies of Venezuelan Bignoniaceae. III. Jour. Wash. Acad. Sci. 18:333-343. 1928.

<sup>10</sup> FERNALD, M. L., *Geocaulon*, a new genus of the Santalaceae. Contrib. Gray Herb. 79. pp. 21-24. 1928.

<sup>11</sup> ROBINSON, B. L., Records preliminary to a general treatment of the Eupatorieae. Contrib. Gray Herb. 80. pp. 1-42. 1928.

<sup>12</sup> JOHNSTON, I. M., Studies in the Boraginaceae. VII. Contrib. Gray Herb. 81. pp. 3-83. 1928.

<sup>13</sup> ———, Some undescribed American Spermatophytes. Contrib. Gray Herb. 88. pp. 85-98. 1928.

STANDLEY<sup>14</sup> has published 15 new species of Rubiaceae from Central America, representing 4 genera. Most of them belong to *Psychotria* and *Palicourea*, each of which contains 6 of the species.

BROWN,<sup>15</sup> in continuation of his studies of *Mesembryanthemum* and allied genera, has described a new genus (*Dicrocaulon*) which includes 6 species.

TIFFANY<sup>16</sup> has published a very full and well organized account of *Bulbochaete* (Oedogoniaceae), which will be a valuable reference paper for students of algae. He recognizes 51 species, 15 varieties, and 7 forms. One new species and one new variety are described, and the 10 plates make visible the characters used.

BUSH<sup>17</sup> has described as a new species (*Quercus neo-ashei*) the Black Jack oak occurring in middle Texas, which heretofore has been included in *Q. marilandica*.

ETHEL M. DOIDGE<sup>18</sup> has published a very detailed "preliminary account" of the rusts of South Africa, in a contribution from the National Herbarium at Pretoria. It includes descriptions, mostly illustrated, of nearly 400 species, many of which are new, included in 23 genera. The large genera are as follows: *Puccinia* (133 species, 29 new), *Aecidium* (72 species, 16 new), *Uromyces* (71 species, 12 new), *Uredo* (30 species, 5 new), and *Ravenellia* (21 species, 5 new).

Miss DOIDGE, in a subsequent contribution,<sup>19</sup> has described 14 new species of South African Ascomycetes, including a new genus (*Stigmatopeltis*).

STENT,<sup>20</sup> in connection with this study of the South African Gramineae, has described 28 species of *Sporobolus*, 10 of which are new.

PHILLIPS<sup>21</sup> has described the following new genera from South Africa: *Pseudoscopia* (Flacourtiaceae), *Keetia* (Rubiaceae), and *Theilera* (Campanulaceae).—J. M. C.

<sup>14</sup> STANDLEY, PAUL C., New plants from Central America. XIII. Jour. Wash. Acad. Sci. 18:273-282. 1928.

<sup>15</sup> BROWN, N. E., *Mesembryanthemum* and allied genera. Jour. Botany 66:138-145. 1928.

<sup>16</sup> TIFFANY, L. H., The algal genus *Bulbochaete*. Trans. Amer. Micr. Soc. 47:131-177. 1928.

<sup>17</sup> BUSH, B. F., *Quercus neo-Ashci*. Bull. Torr. Bot. Club 55:247-250. 1928.

<sup>18</sup> DOIDGE, ETHEL M., A preliminary study of the South African rust fungi. Bothalia 2: 1-228. 1926.

<sup>19</sup> ———, South African Ascomycetes in the National Herbarium. Bothalia 2:229-241. 1927.

<sup>20</sup> STENT, S. M., South African species of *Sporobolus*. Bothalia 2:247-274. 1927.

<sup>21</sup> PHILLIPS, E. P., Description of three new South African plants. Bothalia 2:368, 369. 1927.

## GENERAL INDEX

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